TASK-1 (KCNK3) channels in the lung: from cell biology to clinical implications

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ABSTRACT TWIK-related acid-sensitive potassium channel 1 (TASK-1 encoded by KCNK3) belongs to the family of two-pore domain potassium channels. This gene subfamily is constitutively active at physiological resting membrane potentials in excitable cells, including smooth muscle cells, and has been particularly linked to the human pulmonary circulation. TASK-1 channels are sensitive to a wide array of physiological and pharmacological mediators that affect their activity such as unsaturated fatty acids, extracellular pH, hypoxia, anaesthetics and intracellular signalling pathways. Recent studies show that modulation of TASK-1 channels, either directly or indirectly by targeting their regulatory mechanisms, has the potential to control pulmonary arterial tone in humans. Furthermore, mutations in KCNK3 have been identified as a rare cause of both familial and idiopathic pulmonary arterial hypertension. This review summarises our current state of knowledge of the functional role of TASK-1 channels in the pulmonary circulation in health and disease, with special emphasis on current advancements in the field.

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The family of two-pore domain potassium (K2P) channels

Historical overview

Potassium (K^+) channels constitute the largest group of ion channels in the human genome. They span the membrane of cells, allowing the selective permeation of K^+ ions from one side of the membrane to the other, usually from the inside of the cell to the outside. Their activity is gated by a range of stimuli, including voltage and a variety of physiological and pharmacological mediators. They regulate the excitability of cells and contribute to their resting membrane potential [1, 2]. Mutations in K^+ channel sequences can lead to a variety of clinical disorders exemplifying their physiological importance [3].

K^+ channels are characterised by their exquisite selectivity for K^+ ions, due to a conserved canonical amino acid GYG signature sequence in the selectivity filter of their pore-forming alpha (α) subunits [4]. In addition, many K^+ channel α subunits are associated with auxiliary regulatory subunits. Distinct families of K^+-selective ion channels have been described in almost all living organisms; principally, the voltage-gated K^+ channel (K_V) and calcium-activated K^+ channel (K_{Ca}) family of six transmembrane channel subunits, the inward-rectifier K^+ channel (K_{IR}) family of two transmembrane channel subunits and the two-pore domain K^+ channel (K2P) family of four transmembrane channels subunits [5–8].

The K2P family are the most recent family of K^+ channels to be identified, and their discovery resolved a phenomenon described over 50 years earlier by HODGKIN and HUXLEY [9, 10], of a high resting K^+ conductance present at the plasma membrane that could not be explained by simple passive pores. K2P channels are widely accepted to underlie "leak" or background currents that stabilise the resting membrane potential of neuronal cells, regulating excitability and action potential firing. The first mammalian K2P channel was isolated in 1996, and was named Tandem of pore domains in a Weak Inward rectifying K^+

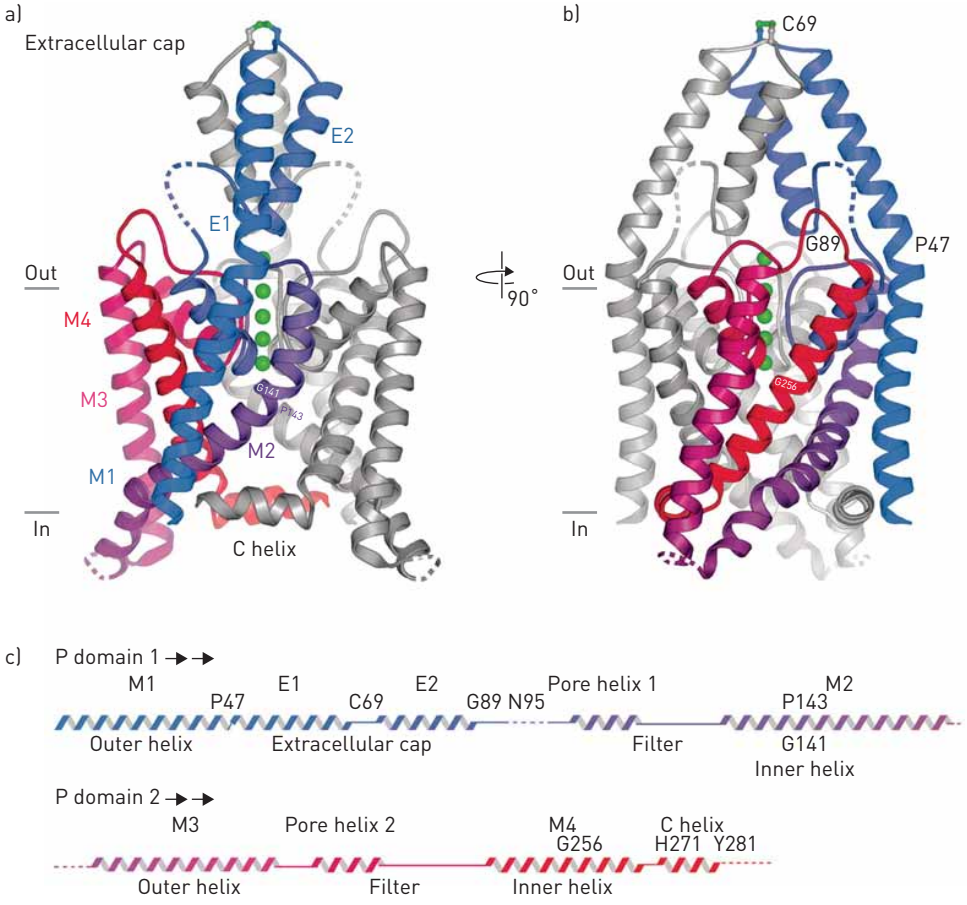

FIGURE 1 Overall structure of the two-pore domain potassium (K2P) channels. a) Tertiary structure of K2P, showing a ribbon representation from the side. One subunit is coloured blue-to-red from the N to the C terminus, and the other subunit is grey. K^+ ions are shown as green spheres. Approximate boundaries of the lipid membrane are shown as horizontal lines. The intersubunit disulfide bond at the apex of the extracellular cap is coloured green. b) An orthogonal view of the channel from the side. c) Secondary structure of K2P coloured according to (a). Dashed lines indicate disordered regions. Reproduced from [123] with permission.

channel or TWIK-1 (KCNK1, $K_{2p}1.1$), based on its general molecular topology consisting of two α-subunits each comprising two pore loop forming (P) domains and four transmembrane segments, which come together as a dimer (figure 1) [11] and, as measured at the time, a functional characteristic of weak inward rectification (but see below).

This discovery was very rapidly followed by the identification of a further 14 mammalian members all sharing the same general TWIK-1 architecture, TREK-1 (KCNK2, $K_{2p}2.1$), TASK-1 (KCNK3, $K_{2p}3.1$), TRAAK (KCNK4, $K_{2p}4.1$), TASK-2 (KCNK5, $K_{2p}5.1$), TWIK-2 (KCNK6, $K_{2p}6.1$), KCNK7, TASK-3 (KCNK9, $K_{2p}9.1$), TREK-2 (KCNK10, $K_{2p}10.1$), THIK-2 (KCNK12, $K_{2p}12.1$), THIK-1 (KCNK13, $K_{2p}13.1$), TASK-5 (KCNK15, $K_{2p}15.1$), TALK-1 (KCNK16, $K_{2p}16.1$), TALK-2 (KCNK17, $K_{2p}17.1$), with TRESK (KCNK18, $K_{2p}18.1$) the final one to be identified in 2003 (figure 2a) [12].

Biophysical properties of the two-pore domain potassium channels

Despite their structural similarities these channels can be further divided into six distinct subfamilies based on their sequence similarity and functional properties (TWIK, TREK, TASK, TALK, THIK and TRESK)

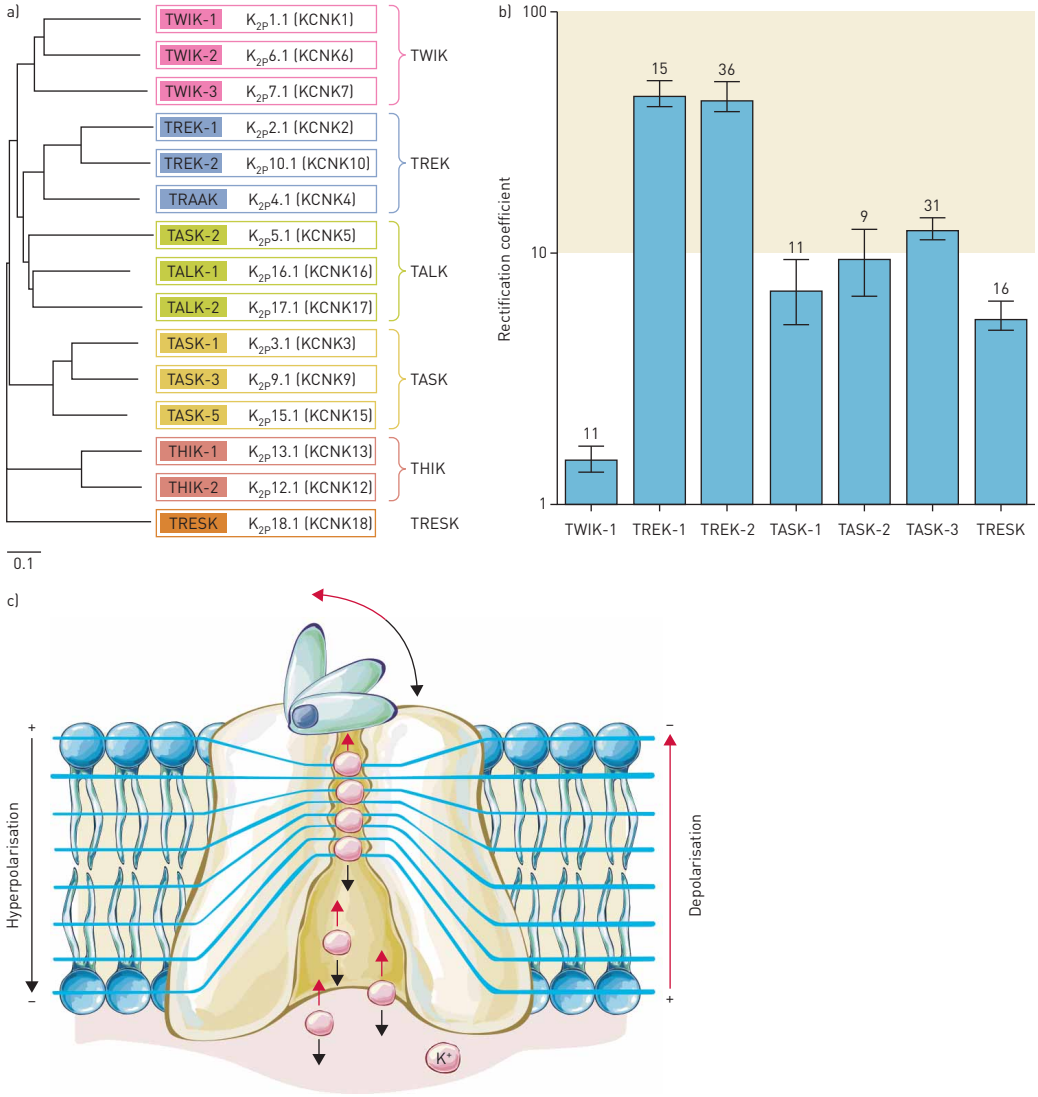

FIGURE 2 Characteristics of human K2P channels. a) Phylogenetic tree of human K2P channels. The nomenclature, of each channel subunit is indicated. The highest region of homology between these subunits is found in the P regions. Six structural and functional subgroups are identified by different colours. b) Rectification coefficients (currents at +100 mV/−100 mV) subsequent to a depolarising pulse to +100 mV (from a holding voltage of −100 mV) are shown for the indicated K2P channels. c) Ion-flux gating of K2P channels. Reproduced from [13] with permission.

(figure 2a). The diversity of these channels are further increased by heteromeric assembly within subfamilies; association with auxiliary subunits; and channel susceptibility to both alternative splicing and alternative translation initiation.

As predicted for a background current following the Goldman–Hodgkin–Katz equation, TWIK-1 was found to be constantly active, time and voltage-independent, with an almost linear current–voltage relationship (figure 2b and c) [11, 13]. The other 14K2P channels, like TWIK-1, do not possess a classical voltage sensor; however, unlike TWIK-1, they display a voltage-dependent conductance increase upon depolarisation and an instantaneous followed by a time-dependent current component [13]. As such, for these K2P channels, at positive and negative voltages equidistant from the reversal potential, there is much more outward current observed than inward current as exemplified by the high rectification coefficients seen in figure 2b compared to those seen for TWIK-1 channels.

How these channels sense voltage in the absence of any canonical voltage sensor domain results from an ion-flux gating mechanism powered by the electrochemical K^+ gradient [13] which may also be regulated directly by many of the other factors which gate K2P channels [14].

Two-pore domain potassium channels in the lung

Potential functions of the K2P family have been studied over the past few years in the lung. In normal human bronchial epithelial cells and at the apical membrane of airway and alveolar epithelial cells multiple KCNK genes that encode K2P channels such as KCNK1, KCNK2, KCNK5 and KCNK6 have been identified [15]. Notably, TWIK-2/KCNK6 appeared to be expressed in cilia, where it could serve as a chemical sensor and improve mucociliary clearance [15]. Stretch-activated K2P channels such as TREK-1/KCNK2 are particularly important in the lung, since they play a central role in mechanotransduction processes. The effects of mechanical stretch on many biological lung functions, including fetal lung growth, surfactant metabolism, extracellular matrix and cytoskeleton turnover, cell proliferation, apoptosis, mediator release and alveolar-capillary permeability, have been recognised for more than two decades. Moreover, mechanical ventilation and oxygen therapy comprise the cornerstones of life-saving interventions for patients with acute respiratory distress syndrome (ARDS) [16].

Alveolar epithelial cells express TREK-1 and in those cells it may play a regulatory role in the development of alveolar epithelial injury. Evidence from the group of A. Schwingshackl and co-workers suggests that K2P channels, especially TREK-1, are important regulators of the inflammatory processes observed in ARDS since they are expressed in lung epithelial cells and macrophages and are regulated by both stretch and hyperoxia [17, 18]. In fact, stimulation of TREK-1-deficient alveolar epithelial cells with TNF-α, decreased IL-6 and RANTES secretion but increased MCP-1 secretion, while KC/IL-8 release was not affected. Furthermore, TREK-1 deficiency accentuated hyperoxia-induced lung injury *in vivo* [19]. The lung injury was evidenced by decreased compliance, increased pulmonary inflammatory infiltrates including neutrophils, macrophages and lymphocytes, and an increase in apoptotic cells in the mouse model. Clinically, this suggests that *in vivo* TREK-1 may play an important role in preventing or modulating moderate hyperoxia-induced lung injury [19]. Taken together, the data support the hypothesis that, in surface epithelial cells, K2P channels contribute to lung inflammation and mucociliary clearance and may be potential therapeutic targets in acute lung injury.

Inward rectifier, voltage-gated delayed rectifier, Ca^{2+}-activated and ATP-sensitive K^+ channels have been shown to regulate the membrane potential in vascular myocytes isolated from a range of small arteries and arterioles [20–22]. Acute contraction of pulmonary arterial smooth muscle cells (PASMCs) is activated, in part, by a K^+ channel inhibition-induced membrane depolarisation and subsequent Ca^{2+} entry through nifedipine-sensitive L-type Ca^{2+} channels (figure 4 [23]). K^+ channel properties (*e.g.* voltage and/or Ca^{2+}-dependent gating) are not well matched; however, to the resting conditions in pulmonary arteries and they make poor candidates for the background K^+ conductance [24–27]. In contrast, the fast-growing family of K2P channels have biophysical and pharmacological properties well suited to a role in mediating background K^+ conductance and resting membrane potential [28, 29]. As their physiological roles emerge, the K2P family of potassium channels may offer promising therapeutic solutions to target pulmonary vascular disease diseases [30].

TASK-1 two-pore domain potassium channel

The TASK family

The TASK (TWIK-related Acid Sensitive K^+) family of K2P is comprised of three members: TASK-1 (KCNK3); TASK-3 (KCNK9) and the non-functional TASK-5. TASK-3 is unusual in that it is the only K2P channel that is genetically imprinted and is exclusively expressed on the maternal allele, with paternal silencing [31]. These channels were traditionally thought to be voltage-independent, openly rectifying and obey the Goldman–Hodgkin–Katz equation (figure 3) [32–36]. However, it is now clear that their

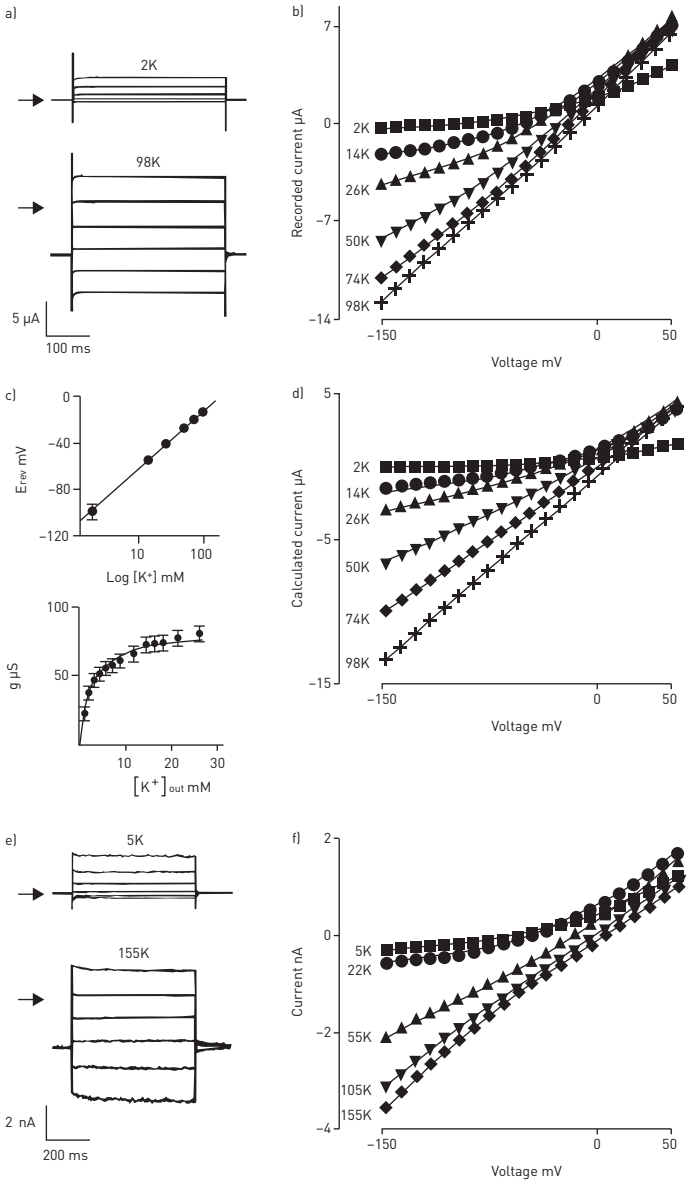

FIGURE 3 Representative recordings illustrating the biophysical properties of TASK-1 channels in *Xenopus* oocytes and COS cells. a) TASK currents recorded from a *Xenopus* oocyte injected with TASK cRNA and elicited by voltage pulses from −150 to +50 mV in 40 mV steps, 500 ms in duration, from a holding potential of −80 mV in low (2 mM K^+) or high K^+ solutions (98 mM K^+). The zero current level is indicated by an arrow. b) Current–voltage relationships. Mean currents were measured over the last 50 ms at the end of voltage pulses from −150 to +50 mV in 10 mV steps as in (a). Modified ND96 solutions containing 2 mM K^+ and 96 mM TMA were used, TMA was then substituted by K^+ to obtain solutions ranging from 2 to 98 mM K^+. TASK currents are not sensitive to external TMA, no changes were observed upon substitution of NaCl by TMA (data not shown). c) Upper panel: reversal potentials of TASK currents as a function of external K^+ concentration (mean±SEM, n=3). Lower panel: slope conductance measured between +10 and +50 mV on current–voltage relationships as in (b), plotted as a function of the external K^+ concentration (mean±SEM, n=3). The mean values were fitted with a hyperbolic function. d) Theoretical current–voltage relationship under the same conditions as in (b), calculated according to the following modified Goldman–Hodgkin–Katz (GHK) current relationship: $I_{K^+} = \bar{P}_{K^+} \cdot ([K^+]_{out}/(K_{0.5} + [K^+]_{out})) \cdot (V_m F^2/RT) \cdot ([K^+]_{in} - [K^+]_{out} \cdot e^{-V_m F/RT})/(1 - e^{-V_m F/RT})$ where I_{K+} is the potassium current, P_{K+} is the apparent permeability for K^+, $K_{0.5}$ the half maximum activation by K^+, $[K^+]_{out}$ and $[K^+]_{in}$ are the external and internal K^+ concentrations, V_m the membrane potential, *F*, *R* and *T* have their usual meanings. The classical GHK relationship has been modified with $[K^+]_{out}/K_{0.5}+[K^+]_{out}$ to take into account the sensitivity of the conductance to external K^+. e) TASK currents recorded from a transfected COS cell and elicited by voltage pulses from −150 to +50 mV in 40 mV steps, 500 ms in duration, from a holding potential of −80 mV, in low (5 mM K+) or high K^+ solutions (155 mM K^+). The zero current level is indicated by an arrow. f) Current–voltage relationships. Mean currents were measured over the last 50 ms at the end of voltage pulses ranging from −150 to +50 mV in 10 mV steps as in (e). Solutions containing 5 mM K^+ and 150 mM TMA were used, TMA was then substituted by K^+ to obtain solutions ranging from 5 to 155 mM K^+. Reproduced from [32] with permission.

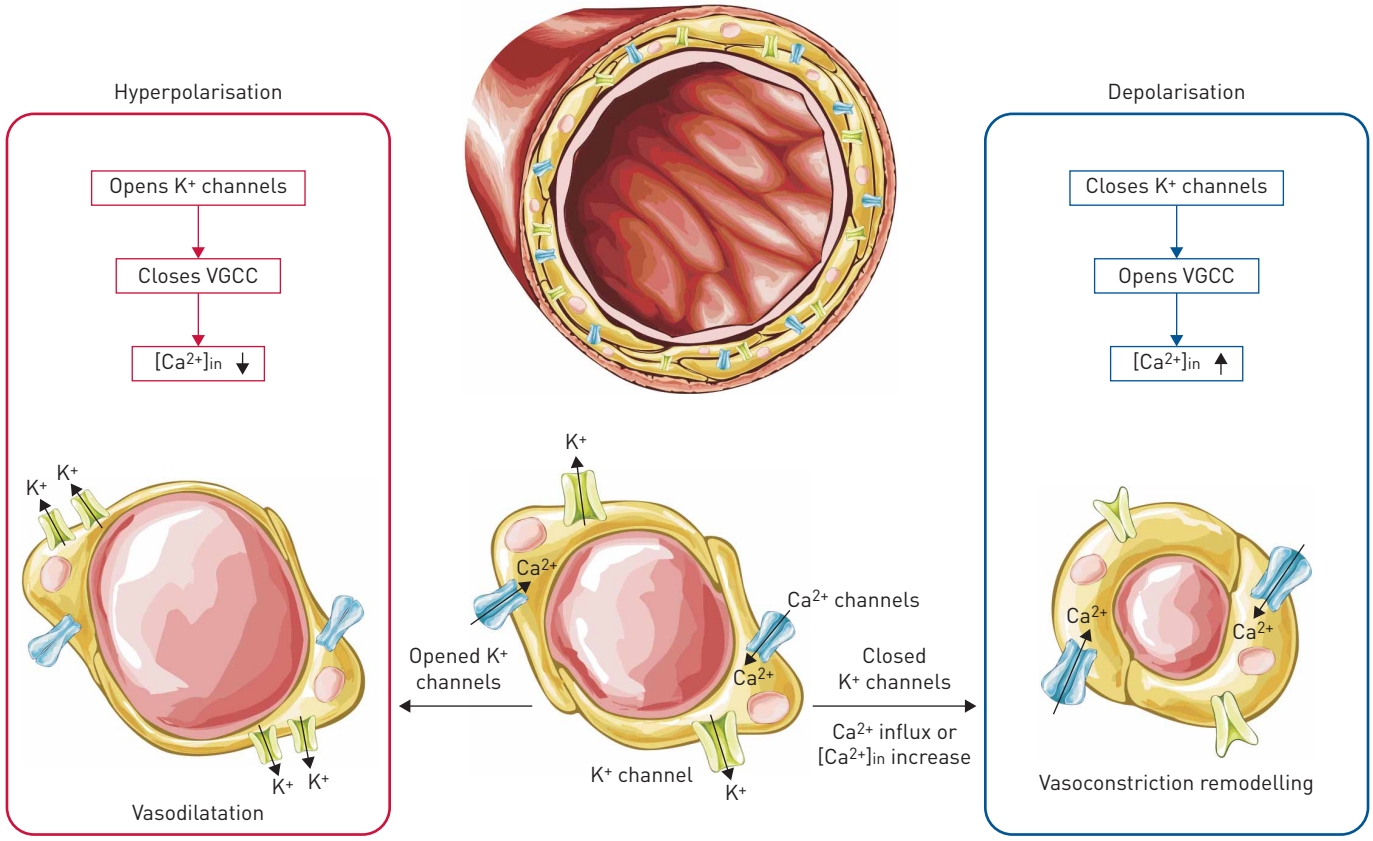

FIGURE 4 Regulation of pulmonary vascular tone by potassium and calcium channels. Channel proteins are indicated by yellow (K^+ channels) and blue (Ca^{2+} channels) membrane structures. Active channels are highlighted by arrows. Left panel: opening of K^+ channels lead to hyperpolarisation of pulmonary artery smooth muscle cells resulting in closure of voltage-sensitive Ca^{2+} channels and subsequent vasodilation. Right panel: acute contraction of pulmonary artery smooth muscle cells is activated in part by K^+ channel inhibition-induced membrane depolarisation and subsequent Ca^{2+} entry through nifedipine-sensitive L-type Ca^{2+} channels.

prominent outward rectification responsible for stabilising the resting membrane potential is a result of a time- and voltage-dependent activation process [13].

Functional diversity of this family is increased by the formation of heterodimers between TASK-1 and TASK-3 [37–42] and, perhaps, with TWIK-1 [43], and by the interaction with auxiliary subunits such as coat protein 1, 14-3-3, p11 and syntaxin-8 [44–49].

They are thought to contribute to the background currents in many neuronal populations throughout the central nervous system, including cerebellar granule neurons, cerebral cortex, the brainstem Pre-Botzinger and retrotrapezoid regions, hippocampal neurons, thalamocortical relay neurons, hypoglossal and spinal cord motor neurons, dorsal vagal neurons (see review by Enyedi and Czirjak [12]). In peripheral tissues high levels of TASK-1 expression have been found in the carotid bodies, in the atrium of the heart; in neuroepithelial bodies of the lung and in PASMCs [25, 50–54].

Regulation of TASK-1 channels and their clinical relevance

Sensitivity of TASK-1 channels to the extracellular pH has attracted attention from the first description of the channel, indeed the channel has been named on the basis of this feature (TWIK-related Acid Sensitive K^+ channel) and it later proved to be a physiologically significant regulatory mechanism [32]. TASK-1 shows about half maximal activity at physiological extracellular pH (7.4). It can be efficiently inhibited or activated by acidosis or alkalosis respectively (figure 5). The peripheral chemoreceptor glomus cells in the carotid body express the heterodimers composed of TASK-1 and TASK-3 in their plasma membrane, and the inhibition of their background K^+ current and the following depolarisation in response to acidification contributes to increased ventilation (for review see [55]). In addition to this, pH regulation of the members of TASK subfamily may also be important in other tissues as in the case of acidosis-induced pulmonary artery smooth muscle contraction in the pulmonary circulation [56]. The pH sensitivity of TASK-1 (and also TASK-3) relies to a great extent on the protonation of a single histidine residue (H98 in

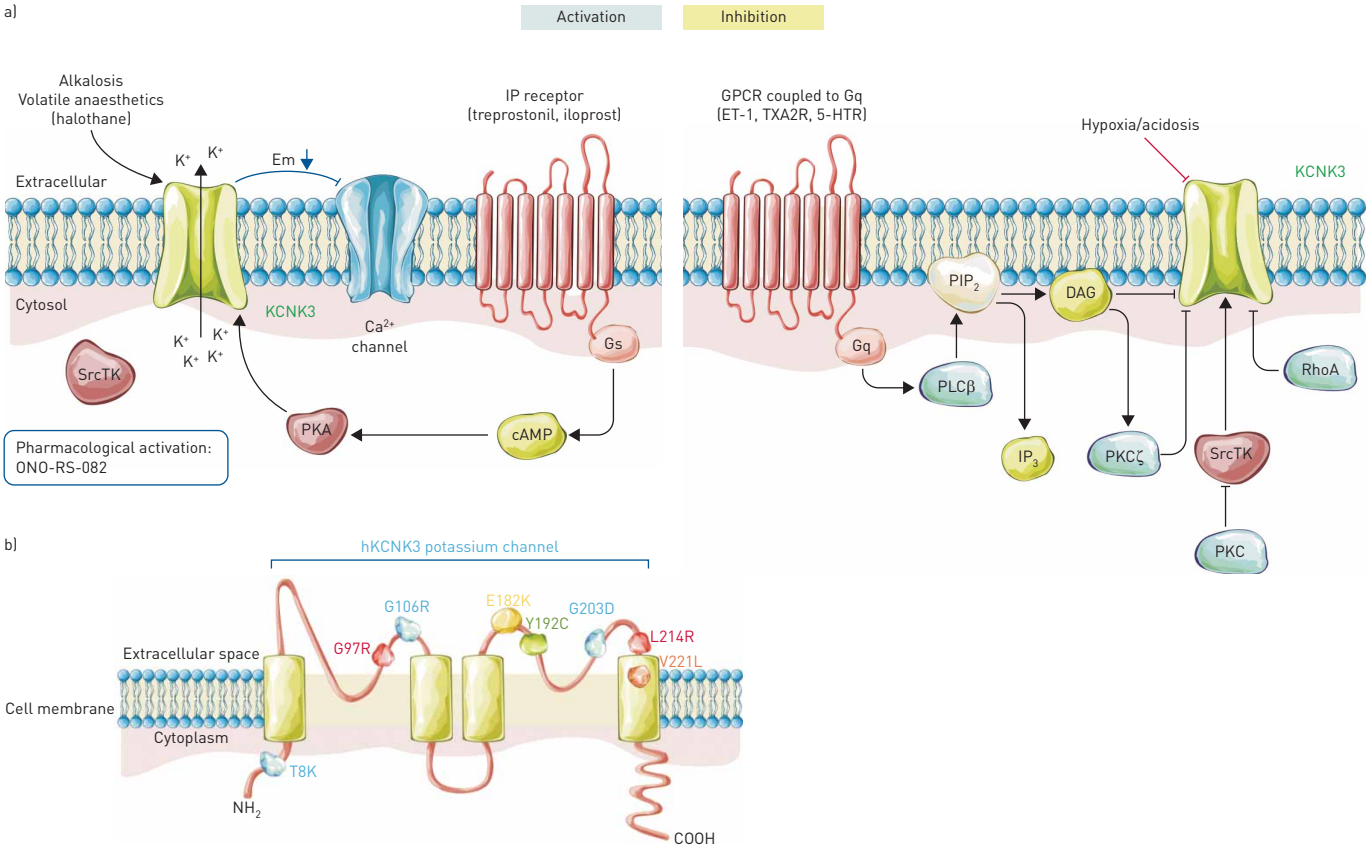

FIGURE 5 TASK-1 in pulmonary arterial smooth muscle cells. a) Regulation of TASK-1 in pulmonary arterial smooth muscle cells. ET-1: endothelin-1; Em: membrane potential; PIP2: phosphatidylinositol-4,5-biphosphate; IP3: inositol-1,4,5-triphosphate; DAG: diacylglycerol; PLC: phospholipase C; PKC: protein kinase C; SrcTK: Src family tyrosine kinase; TXA2R: thromboxane A2 receptor; 5-HTR: 5-hydroxytryptamine receptor; PDGFR: platelet-derived growth factor receptor; FGFR: fibroblast growth factor receptor; RTK: receptor tyrosine kinases. b) Topological analysis of the human KCNK3/TASK-1 channel. Positions indicating the mutations identified by Ma *et al.* [90] and Navas Tejedor *et al.* [116].

TASK-1) located in the first extracellular loop of the channel [36, 57]. H98 is not conserved in TASK-2, thus other residues are responsible for the pH-sensitivity of TASK-2, which belongs to the TALK but not the TASK subfamily of K2P channels [58]. H98 together with the negatively charged glutamate E70 also contribute to the inhibition of human TASK-3 by Zn^{2+}; however, human TASK-1 is much less inhibited by the divalent ion, because it contains lysine instead of glutamate at position 70 [59]. It has been reported that acidification also interferes with the K^+ selectivity of TASK-1 in addition to the inhibition of the current, as a mechanism resulting in further depolarisation [60].

TASK-1 is widely regulated by Gq-coupled receptors. This has been demonstrated in a wide variety of native cell types. TASK-1 or the heterodimeric TASK-1/TASK-3 channels were found to be inhibited by several Gq-coupled receptor types in motoneurons [39, 61], cerebellar granule neurons [62], thalamocortical neurons [63, 64] and adrenal glomerulosa cells [65, 66]. Whereas different neurotransmitters enhance neuronal excitability through the inhibition of TASK channels, the inhibition of the channels by circulating angiotensin II in the glomerulosa cells results in increased production of aldosterone and the consequent retention of Na^+ and water [66].

In accordance with the general importance of receptor-mediated inhibition of these channels, the mechanism of this regulation has been extensively examined in heterologous expression systems. There has been a long lasting debate regarding the steps leading to the altered channel activity. Early results revealed the significant role of canonical signalling pathways (InsP3, calcium and protein kinase C). It has been reported that Gαq can directly bind to and inhibit TASK-1 [67]. However, a more commonly held view, currently, is that the activation of phospholipase C (PLC) is required for inhibition of the channels [68–70]. Originally it has been suggested that the breakdown and reduced steady state level of PIP2 was responsible for the inhibition [68], particularly as PIP2 containing liposomes, or its water soluble analogues, were found to activate TASK-1 in inside-out membrane patches [71]. However, later it was demonstrated that the depletion of PIP2 in living cells by coexpressed lipid phosphatases does not affect

TASK-1 activity, and the lipid end product diacylglycerol (DAG) of PLC enzyme mediates the effect [72]. In addition to the PLC pathway of regulation, other mechanisms were also reported to inhibit TASK-1 in PASMCs. For example, Rho-kinase inhibits the channel by phosphorylating threonine 393 of TASK-1 in response to endothelin receptor activation [73], and Src tyrosine kinase also crucially controls TASK-1 channel activity [74]. These mechanisms clearly require further investigation and they indicate that parallel cell type specific signalling pathways may regulate TASK-1 activity in a complex manner as is seen for Gαq mediated regulation of other potassium channels.

TASK-1 plays a key role in the sensing of hypoxic stimuli. Inhibition of TASK-1 by hypoxia has been extensively studied in dedicated chemosensory (glomus type I) cells [41, 75] (for review see [76]) and the importance of hypoxia related regulation was also clearly demonstrated in pulmonary resistance vessels [52, 56] (for review see [77]). Even before the discovery of TASK, inhibition of a leak potassium current was known to be a major factor leading to depolarisation in glomus cells of the carotid body. Later, these leak conductance were identified as TASK-1/TASK-3 heterodimers and TASK-1 homodimers, the latter being more sensitive to hypoxia [40, 41]. The effects of hypoxia on channel activity are still not well defined. Given the influence of metabolic changes on ion channel regulation, reduced oxygen tension related to mitochondrial respiration, the contribution of the cytoplasmic ATP concentration, AMP kinase, H_2S and CO have been investigated to uncover their role on the matter. The theory of indirect regulation is supported by the observed rapid rundown of channel activity during excised patch experiments following the removal of TASK channels from their natural environment. The regulatory elements may be active in particular oxygen sensitive tissues (glomus cells, pulmonary arterioles and neuroepithelial bodies), while their absence may explain the lack of response to hypoxia elsewhere and the failure to reproduce this type of regulation in heterologous expression systems.

Volatile anaesthetics activate most K2P channels and TASK-1 is also a target of halothane, isoflurane and sevoflurane [78, 79] (figure 5). The activation of TASK-1 in motoneurons contributes to the immobilising effects of inhalational anaesthetics [80]. Local anaesthetics, *e.g.* lidocaine and bupivacaine, non-specifically inhibit TASK-1 at relatively high concentration [81]. Anandamide and methanandamide are nonselective blockers of TASK-1 and TASK-3 [39, 82]; however, it remains to be established whether TASK channels mediate cannabinoid receptor-independent effects of these endocannabinoids. The respiratory stimulants, doxapram, PKTHPP and A1899, inhibit TASK-1/TASK-3 channels and may act in the carotid body [40, 83, 84]; these are potential therapeutic agents in sleep apnoea, and it has been known for decades that doxapram can reverse human respiratory depression induced by morphine. Another TASK-1/TASK-3 inhibitor, A293, which has a slight preference for TASK-1, was used to demonstrate TASK expression in rat cardiac myocytes [85]. Later, TASK-1/TASK-3 channels were also detected in human atrial cardiac myocytes [42, 86]. Upregulation of TASK-1 was reported in patients with chronic atrial fibrillation [87, 88]. Interestingly, loss of function mutations in TASK-1 were also found to be associated with atrial fibrillation [89].

Inactivating mutations of TASK-1 have also been demonstrated to cause pulmonary arterial hypertension (PAH) in patients [90], raising the question whether a drug used for chronic inhibition of TASK channel in atrial fibrillation would cause pulmonary hypertension as a side-effect and, alternatively, whether a TASK activator for the treatment of PAH could result in atrial fibrillation. Furthermore, TASK-1 is also highly expressed in human adrenal glomerulosa cells together with the inwardly rectifying K^+ channel Kir3.4 encoded by the KCNJ5 gene [91, 92]. Although the mutations of KCNJ5 resulting in increased Na^+ conductance are responsible for the clinical cases of primary hyperaldosteronism [93], pharmacological modulation of TASK-1 may also influence aldosterone production and the salt water balance. This idea is also supported by the primary hyperaldosteronism evoked by TASK-1 gene knockout in rodent models [66, 94], and by the association of human TASK-1 (KCNK3) variants with hypertension and high plasma aldosterone levels [95]. In addition, knockout mice lacking TASK-1 channels are characterised by impaired carotid body chemoreceptor function [96]. Pharmacological interventions and therapeutic modalities have to differentially target these overlapping and important physiological functions of TASK-1 to avoid adverse effects.

TASK-1 channels in the mammalian lung circulation – interspecies differences

It is important to realise that the pulmonary circulation differs from systemic circulation with regards to vasoregulation under hypoxaemia, blood pressure regulation and anatomy. 1) In contrast to the systemic circulation, hypoxaemia leads to vasoconstriction of small resistance arteries in the pulmonary circulation. This physiological response is called as hypoxic pulmonary vasoconstriction and responsible for optimising the matching of perfusion and ventilation and preventing arterial hypoxaemia [97, 98]. 2) The pulmonary circulation lacks of regulation by central nervous control mechanism. 3) Finally, the large, muscular pulmonary arteries directly merge into small, partially muscular vessels designed for a very low

perfusion resistance. In the systemic circulation, the arterioles usually have a continuous thick layer of smooth muscle cells that have a high perfusion resistance.

Ion channels play a central role for the regulation of the pulmonary vascular tone and for mediating the effect of physical and chemical stimuli. They can be considered the executive limb of the response. However, the distribution of the ion channels in the pulmonary circulation differs from systemic circulation. It is often questioned whether the K^+ channels are active at sufficiently negative potentials to set the resting membrane potential of PASMCs and which K^+ channels could regulate pulmonary vascular tone [52, 54, 56, 99–104]. Effective modulation of membrane potential by K^+ channels has been shown in rat PASMCs: overexpression of voltage-gated K^+ channels (Kv1.5) led to significant hyperpolarisation *in vitro* and reduction of pulmonary vascular resistance in hypoxia-induced pulmonary hypertension in this species *in vivo* [105, 106].

Recent evidence supports the role of TASK-1 in controlling resting membrane potential in PASMCs. Its voltage-independent gating makes it a good candidate for the maintenance of resting membrane potential in cells, where resting membrane potential has to be kept low in order to keep calcium influx through voltage-gated calcium channels negligible. TASK-1 is expressed in rabbit [52], mouse [100], rat [54, 101] and human [56] PASMCs and the non-inactivating K^+ current (I_{KN}), proposed to be mediated by TASK-1, shows the distinguishing features of the channel [26, 37, 76, 77]. Most importantly, from the TASK family only TASK-1 is present in human PASMCs, making it particularly susceptible in the human pulmonary circulation [56]. Despite evidence that K^+ channels control resting membrane potential, investigations during the last decades confirmed significant interspecies differences in resting potential in PASMCs and, thus, highlighted important interspecies variability in the physiology of pulmonary arteries. While in rat, rabbit or human PASMCs the resting membrane potential is around −50 mV or less, mouse PASMCs have a resting potential closed to −30 mV. Accordingly, the amplitude of the I_{KN} is in a similar range in rat, rabbit or human PASMC. In contrast, I_{KN} in mice is hardly detectable and lacks of the distinguishing features of I_{KN} in other species [100]. Furthermore, the resting membrane potential or I_{KN} do not differ between PASMCs obtained from wild-type and TASK-1/3 knockout animals, confirming the lack of functional TASK-1 in the pulmonary arteries in mice. Thus, evidence is accumulating that where the resting membrane potential of PASMC is depolarised, I_{KN} is absent and TASK-1 not required for the normal pulmonary arterial function [100]. More recent work shows that TASK-1 channels do not have a role in initiating hypoxic pulmonary hypertension in murine intrapulmonary arteries [107]. Indeed, in mice, TASK-1 function seems to be replaced by other K2P channels. Accordingly, TWIK-2 (KCNK6) deficient mice spontaneously develop pulmonary hypertension [108]. In contrast, TASK-1 is functionally expressed in rats and KCNK3 inhibition with A293 was shown to predispose pulmonary arteries to constrict [109]. Furthermore, TASK-1 expression and function were reduced in the monocrotaline-induced pulmonary hypertension model in rats. Together, these findings question the use of mice as a model to investigate human pulmonary vascular physiology and especially the functional roles of K^+ channels.

TASK-1 in the human pulmonary circulation and its relevance for the human disease

Mutations, downregulation or inhibition of K^+ channels has been proposed to contribute to pulmonary vascular remodelling in man, resulting in pulmonary hypertension [110, 111]. Pulmonary hypertension is defined by a rise of the mean pulmonary artery pressure (mPAP) of 25 mmHg or more due to a progressive increase of pulmonary vascular resistance to a level where the right ventricle compensates, through right ventricular hypertrophy, and fails when it is unable to fulfil the rise of afterload. Major advances in the understanding of pulmonary hypertension have led to the current classification in which the pulmonary hypertension diseases are grouped into five categories according to cause and therapeutic strategy [112]. The diverse and complex mechanisms underlying the pathogenesis of PAH (group 1 pulmonary hypertension) include vasoconstriction, *in situ* thrombosis, progressive vascular remodelling of the small pulmonary arteries (<500 µm), an excess of vasoconstrictors and a parallel deficiency of vasodilating mediators [113].

Reduced K^+ channel activity in PASMCs promotes cell proliferation, resistance to apoptosis and vasoconstriction contributing to vascular remodelling [110]. Although a wide-range of K^+ channels have been found in human PASMC, for PAH only the roles of Kv1.5 and TASK-1 channels have been confirmed using human pulmonary arteries and primary human PASMCs. The first ion channel reported to be linked to pulmonary hypertension was Kv1.5 [114]. Its reduced expression was detected in PAH. Moreover, single-nucleotide polymorphisms in KCNA5 (Kv1.5) have been identified in idiopathic PAH patients leading to decrease KCNA5 function [115]. However, the strong voltage dependent activation of the channel and the lack of hyperpolarising periods in order to ensure the recovery in PASMC, results in the accumulation of the channels in the inactivated state and thus, challenges the significance of Kv1.5 in the pulmonary circulation.

In 2013, five different mutations were identified in KCNK3 gene (TASK-1) in PAH patients (figure 6). Heterozygous KCNK3 mutations were observed in 1.3% sporadic and 3.2% heritable PAH patients. Patch-clamp experiments demonstrated a loss of function in all five identified mutations [90]. More recently, two additional KCNK3 mutations have been identified in Spanish cohort of PAH patients. Interestingly, this report described the first case of PAH occurring in a patient with homozygous KCNK3 mutations associated with aggressive form of PAH [116]. To date eight different KCNK3 mutations have been described in PAH patients (figure 6). Thus, KCNK3 mutations are the first channelopathies known to cause PAH to date [90].

In human PASMC an siRNA approach against KCNK3 demonstrated that KCNK3 contributes to the resting membrane potential suggesting a crucial role of KCNK3 channels in the regulation of pulmonary vascular tone. In addition, TASK-1 is sensitive to hypoxia and activated by treprostinil, a stable analogue of prostacyclin, *via* a protein kinase (PK) A-dependent pathway, representing an important mechanism of the vasorelaxing properties of prostanoids [56]. Moreover, another report revealed that KCNK3 expression was reduced in idiopathic, as well as heritable PAH patients due to BMPR2 mutations (at mRNA and protein levels in lung and isolated pulmonary arteries). In agreement with the reduced expression of KCNK3, patch-clamp experiments showed a severe decrease of A293- (specific KCNK3 inhibitor) sensitive current function in cultured PASMC from idiopathic PAH patients compared to controls [109]. These results suggest that KCNK3 loss of function or decreased expression is a hallmark of PAH [109].

Confirming the crucial role of KCNK3 channels in PAH pathogenesis, endothelin-1, a potent vasoconstrictor for vascular remodelling, has been shown to inhibit KCNK3 *via* Rho kinase [73] and *via* a protein kinase (PK) C-dependent pathway in human PASMCs (figure 5) [117]. Increased expression of endothelin-1 level has been found in pulmonary arteries of PAH patients [118]. However, KCNK3 function could be inhibited by downstream signalling of other G-protein-coupled receptors as diacylglycerol homeostasis directly inhibits KCNK3 [72]. Interestingly, aminorex, fenfluramine or selective serotonin reuptake inhibitors, drugs associated with an increased risk for the development of PAH act *via* these pathways [119]. In addition, the non-receptor tyrosine kinase activity seems to be essential for the function of TASK-1 since targeted inhibition of c-Src by siRNA reduces TASK-1 current in human PASMCs [74]. The recently reported association of severe PAH with dasatinib, the c-Src kinase inhibitor used in the treatment of chronic myelogenous leukaemia suggests a direct and specific effect of s-Src inhibition on pulmonary vessels [120]. It is noteworthy, that in PAH patients the protein expression of the non-receptor tyrosine kinase c-Src is significantly reduced in the lung [121]. These findings demonstrate the key role of TASK-1 in many different pathways leading to PAH.

Beyond the direct effects of the TASK-1 inhibition on the vasoreactivity in the pulmonary circulation, the role of KCNK3 channel in the proliferation/apoptosis balance of human PASMC remained unknown. Recent investigations demonstrated that *in vivo* chronic KCNK3 inhibition in rats induced an exaggerated proliferation of pulmonary artery endothelial cells, PASMCs and adventitial fibroblasts, which could initiate or promote the development of pulmonary hypertension [109]. In addition, the membrane potential of PASMC from KCNK3 deficient rats, generated by using CRISPR-Cas9 technology, are significantly depolarised and the mutation induced distal neomuscularisation, abnormal pulmonary artery vasoreactivity and elevated mean right ventricular systolic pressures, confirming that KCNK3 loss of function is a key event in PAH pathogenesis [122].

Conclusion and future directions

Both preclinical and clinical studies strongly support TASK-1 channels as important players in the pathology of pulmonary vascular diseases. As TASK-1 channels regulate resting membrane potential in human PASMC and consequently low pulmonary vascular tone, in order to achieve maximum vasodilation under pathophysiological conditions, restoring TASK-1 channel function channel is a viable therapeutic approach. Although, TASK-1 has an accessible cell surface location and considerable tissue-defined distribution, TASK-1 remains underexploited as a target in drug discovery. This may be due to a number of factors. There is a limitation of primary human lung tissue and the drug delivery has to be tissue-specific to avoid unpredictable side-effects, even for specific activators. High-throughput screening methods for ion channel targets lack temporal resolution over a physiologically relevant range and manual patch-clamping is time-consuming. In addition, channel modifiers often need to bind to relatively inaccessible sites within the channel pore or to accessory or regulatory domains. *In silico* modelling and advances in structural biology techniques to crystallise channel proteins in complex with accessory subunits may reveal key interaction sites and interfaces for drug design. Indeed, modulating channel behaviour, rather than directly targeting the ion-conducting subunit may ultimately be a more fruitful approach. In this context, the combination therapy for cystic fibrosis using lumacaftor and ivacaftor might suggest a novel therapeutic direction. In the case of cystic fibrosis, lumacaftor increases expression of the

cystic fibrosis transmembrane conductance regulator Cl^- channel at the cell surface and ivacaftor increases its open probability. The need for novel, effective ion channel modulators exists but now the challenge is to match therapeutic strategy with innovative design.

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