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

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## Abstract

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5 TWIK-related acid-sensitive potassium channel 1 (TASK-1 encoded by KCNK3) belongs to  
6 the family of two-pore domain potassium channels. This gene subfamily is constitutively  
7 active at physiological resting membrane potentials in excitable cells, including smooth  
8 muscle cells and has been especially linked to the human pulmonary circulation. TASK-1  
9 channels are sensitive to a wide array of physiological and pharmacological mediators that  
10 affect their activity such as unsaturated fatty acids, extracellular pH, hypoxia, anaesthetics  
11 and intracellular signalling pathways. Recent studies show that modulation of TASK-1  
12 channels either directly or indirectly by targeting their regulatory mechanisms has the  
13 potential to control pulmonary arterial tone in humans. Furthermore, mutations in KCNK3  
14 have been identified as a rare cause of both familial and idiopathic pulmonary arterial  
15 hypertension. This review summarises our current state of knowledge of the functional role of  
16 TASK-1 channels in the pulmonary circulation in health and disease, with special emphasis  
17 on current advancements in the field.  
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## The family of two-pore domain potassium (K<sub>2</sub>P) channels

### *Historical overview*

Potassium (K<sup>+</sup>) channels constitute the largest group of ion channels in the human genome. They span the membrane of cells, allowing the selective permeation of K<sup>+</sup> 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<sup>+</sup> channel sequences can lead to a variety of clinical disorders exemplifying their physiological importance [3].

K<sup>+</sup> channels are characterised by their exquisite selectivity for K<sup>+</sup> 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<sup>+</sup> channel α subunits are associated with auxiliary regulatory subunits. Distinct families of K<sup>+</sup>-selective ion channels have been described in almost all living organisms; principally, the voltage-gated K<sup>+</sup> channel (K<sub>V</sub>) and calcium-activated K<sup>+</sup> channel (K<sub>Ca</sub>) family of 6 transmembrane channel subunits, the inward-rectifier K<sup>+</sup> channel (K<sub>IR</sub>) family of two transmembrane channel subunits and the two-pore domain K<sup>+</sup> channel (K<sub>2</sub>P) family of four transmembrane channels subunits [5-8].

The K<sub>2</sub>P family are the most recent family of K<sup>+</sup> channels to be identified and their discovery resolved a phenomenon described over 50 years earlier by Hodgkin and Huxley, of a high resting K<sup>+</sup> conductance present at the plasma membrane that could not be explained by simple passive pores [9,10]. K<sub>2</sub>P channels are widely accepted to underlie “leak” or background currents that stabilize the resting membrane potential of neuronal cells, regulating excitability and action potential firing. The first mammalian K<sub>2</sub>P channel was isolated in 1996, and was named **T**andem of pore domains in a **W**eak **I**nward rectifying **K**<sup>+</sup> channel or TWIK-1 (KCNK1, K<sub>2p</sub>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 [11] (see Figure 1) and, as measured at the time, a functional characteristic of weak inward rectification (but see below).

### **Figure 1**

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<sub>2p</sub>2.1), TASK-1 (KCNK3, K<sub>2p</sub>3.1), TRAAK (KCNK4, K<sub>2p</sub>4.1), TASK-2 (KCNK5, K<sub>2p</sub>5.1), TWIK-2 (KCNK6, K<sub>2p</sub>6.1), KCNK7, TASK-3 (KCNK9, K<sub>2p</sub>9.1), TREK-2 (KCNK10, K<sub>2p</sub>10.1), THIK-2 (KCNK12, K<sub>2p</sub>12.1), THIK-1 (KCNK13, K<sub>2p</sub>13.1), TASK-5 (KCNK15, K<sub>2p</sub>15.1), TALK-1

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3 (KCNK16, K<sub>2p</sub>16.1), TALK-2 (KCNK17, K<sub>2p</sub>17.1), with TRESK (KCNK18, K<sub>2p</sub>18.1) the final  
4 one to be identified in 2003 [12] (see Figure 2a).  
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## 6 **Figure 2**

### 10 ***Biophysical properties of the two-pore domain potassium channels***

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12 Despite their structural similarities these channels can be further divided into six distinct  
13 subfamilies based on their sequence similarity and functional properties (TWIK, TREK,  
14 TASK, TALK, THIK and TRESK, Figure 2a). The diversity of these channels are further  
15 increased by heteromeric assembly within subfamilies; association with auxiliary subunits;  
16 and channel susceptibility to both alternative splicing and alternative translation initiation.  
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19 As predicted for a background current following the Goldman-Hodgkin-Katz equation,  
20 TWIK-1 was found to be constantly active, time and voltage-independent, with an almost  
21 linear current-voltage relationship [11,13] (see Figure 2b and c). The other 14 K2P channels,  
22 like TWIK-1, do not possess a classical voltage-sensor, however, unlike TWIK-1, they display  
23 a voltage-dependent conductance increase upon depolarisation and an instantaneous  
24 followed by a time-dependent current component [13]. As such, for these K2P channels, at  
25 positive and negative voltages equidistant from the reversal potential, there is much more  
26 outward current observed than inward current as exemplified by the high rectification  
27 coefficients seen in Figure 2c compared to that seen for TWIK1 channels.  
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30 How these channels sense voltage in the absence of any canonical voltage sensor  
31 domain results from an ion-flux gating mechanism powered by the electrochemical K<sup>+</sup>  
32 gradient [13] which may also be regulated directly by many of the other factors which gate  
33 K2P channels [14].  
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### 42 ***Two-pore domain potassium channels in the lung***

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44 Potential functions of the K2P family have been studied over the last few years in the lung. In  
45 normal human bronchial epithelial cells and at the apical membrane of airway and alveolar  
46 epithelial cells multiple KCNK genes that encode K2P channels such as KCNK1, KCNK2,  
47 KCNK5, and KCNK6 have been identified [15]. Notably, TWIK-2/KCNK6 appeared to be  
48 expressed in cilia, where it could serve as a chemical sensor and improve mucociliary  
49 clearance [15]. Stretch-activated K2P channels such as TREK-1/KCNK2 are particularly  
50 important in the lung, since they play a central role in mechanotransduction processes. The  
51 effects of mechanical stretch on many biological lung functions, including fetal lung growth,  
52 surfactant metabolism, extracellular matrix and cytoskeleton turnover, cell proliferation,  
53 apoptosis, mediator release, and alveolar-capillary permeability, have been recognized for  
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3 over two decades. Moreover, mechanical ventilation and oxygen therapy comprise the  
4 cornerstones of life-saving interventions for patients with acute respiratory distress syndrome  
5 (ARDS) [16].  
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7 Alveolar epithelial cells express TREK-1 and in those cells it may play a regulatory  
8 role in the development of alveolar epithelial injury. Evidence from the Schwingshackl group  
9 suggests that K2P channels, especially TREK-1, are important regulators of the inflammatory  
10 processes observed in ARDS since they are expressed in lung epithelial cells and  
11 macrophages and are regulated by both stretch and hyperoxia [17,18]. In fact, stimulation of  
12 TREK-1-deficient alveolar epithelial cells with TNF- $\alpha$ , decreased IL-6 and RANTES secretion  
13 but increased MCP-1 secretion, while KC/IL-8 release was not affected. Furthermore, TREK-  
14 1 deficiency accentuated hyperoxia-induced lung injury *in vivo* [19]. The lung injury was  
15 evidenced by decreased compliance, increased pulmonary inflammatory infiltrates including  
16 neutrophils, macrophages, and lymphocytes, and an increase in apoptotic cells in the mouse  
17 model. Clinically, this suggests that *in vivo* TREK-1 may play an important role in preventing  
18 or modulating moderate hyperoxia-induced lung injury [19]. Taken together, the data support  
19 the hypothesis that, in surface epithelial cells, K2P channels contribute to lung inflammation  
20 and mucociliary clearance and may be potential therapeutic targets in acute lung injury.  
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23 Inward rectifier, voltage-gated delayed rectifier, Ca<sup>2+</sup>-activated and ATP-sensitive K<sup>+</sup>  
24 channels have been shown to regulate the membrane potential in vascular myocytes isolated  
25 from a range of small arteries and arterioles [20-22]. Acute contraction of pulmonary arterial  
26 smooth muscle cells (PASMCs) is activated, in part, by a K<sup>+</sup> channel inhibition-induced  
27 membrane depolarization and subsequent Ca<sup>2+</sup> entry through nifedipine-sensitive L-type  
28 Ca<sup>2+</sup> channels (Figure 4 [23]). K<sup>+</sup> channel properties (e.g voltage and/or Ca<sup>2+</sup>-dependent  
29 gating) are not well matched, however, to the resting conditions in pulmonary arteries and  
30 they make poor candidates for the background K<sup>+</sup> conductance [24-27]. In contrast, the fast-  
31 growing family of K2P channels have biophysical and pharmacological properties well suited  
32 to a role in mediating background K<sup>+</sup> conductance and resting membrane potential [28,29].  
33 As their physiological roles emerge, the K2P family of potassium channels may offer  
34 promising therapeutic solutions to target pulmonary vascular disease diseases [30].  
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#### 48 **Figure 4**

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52 TASK-1 two-pore domain potassium channel  
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56 ***The TASK family***  
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3 The TASK (TWIK-related Acid Sensitive K<sup>+</sup>) family of K<sub>2</sub>P is comprised of three members:  
4 TASK-1 (KCNK3); TASK-3 (KCNK9) and the non-functional TASK-5. TASK-3 is unusual in  
5 that it is the only K<sub>2</sub>P channel that is genetically imprinted and is exclusively expressed on  
6 the maternal allele, with paternal silencing [31]. These channels were traditionally thought to  
7 be voltage-independent, openly rectifying and obey the Goldman-Hodgkin-Katz equation [32-  
8 36] (Figure 3). However, it is now clear that their prominent outward rectification responsible  
9 for stabilising the resting membrane potential is a result of a time- and voltage-dependent  
10 activation process [13].  
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### 15 **Figure 3**

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18 Functional diversity of this family is increased by the formation of heterodimers  
19 between TASK-1 and TASK-3 [37-42] and, perhaps, with TWIK-1 [43], and by the interaction  
20 with auxiliary subunits such as coat protein 1 (COP-1), 14-3-3, p11 and syntaxin-8 [44-49].  
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23 They are thought to contribute to the background currents in many neuronal  
24 populations throughout the central nervous system, including cerebellar granule neurons,  
25 cerebral cortex, the brainstem Pre-Botzinger and retrotrapezoid regions, hippocampal  
26 neurons, thalamocortical relay neurons, hypoglossal and spinal cord motor neurons, dorsal  
27 vagal neurons, (see review by Enyedi & Czirjak [12]). In peripheral tissues high levels of  
28 TASK-1 expression have been found in the carotid bodies, in the atrium of the heart; in  
29 neuroepithelial bodies of the lung and in pulmonary artery smooth muscle cells (PASMCs)  
30 [25,50-54].  
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### 37 ***Regulation of TASK-1 channels and their clinical relevance***

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39 Sensitivity of TASK-1 channels to the extracellular pH has attracted attention from the  
40 first description of the channel, indeed the channel has been named on the basis of this  
41 feature (TWIK-related Acid Sensitive K<sup>+</sup> channel) and it later proved to be a physiologically  
42 significant regulatory mechanism [32]. TASK-1 shows about half maximal activity at  
43 physiological extracellular pH (7.4). It can be efficiently inhibited or activated by acidosis or  
44 alkalosis respectively (Figure 5). The peripheral chemoreceptor glomus cells in the carotid  
45 body express the heterodimers composed of TASK-1 and TASK-3 in their plasma  
46 membrane, and the inhibition of their background K<sup>+</sup> current and the following depolarization  
47 in response to acidification contributes to increased ventilation (for review see [55]). In  
48 addition to this, pH regulation of the members of TASK subfamily may also be important in  
49 other tissues as in the case of acidosis-induced pulmonary artery smooth muscle contraction  
50 in the pulmonary circulation [56]. The pH sensitivity of TASK-1 (and also TASK-3) relies to a  
51 great extent on the protonation of a single histidine residue (H98 in TASK-1) located in the  
52 first extracellular loop of the channel [36,57]. H98 is not conserved in TASK-2, thus other  
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3 residues are responsible for the pH-sensitivity of TASK-2, which belongs to the TALK but not  
4 the TASK subfamily of K<sup>2P</sup> channels [58]. H98 together with the negatively charged  
5 glutamate E70 also contribute to the inhibition of human TASK-3 by Zn<sup>2+</sup>, however, human  
6 TASK-1 is much less inhibited by the divalent ion, because it contains lysine instead of  
7 glutamate at position 70 [59]. It has been reported that acidification also interferes with the K<sup>+</sup>  
8 selectivity of TASK-1 in addition to the inhibition of the current, as a mechanism resulting in  
9 further depolarization [60].  
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#### 14 **Figure 5**

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18 TASK-1 is widely regulated by Gq-coupled receptors. This has been demonstrated in  
19 a wide variety of native cell types. TASK-1 or the heterodimeric TASK-1/TASK-3 channels  
20 were found to be inhibited by several Gq-coupled receptor types in motoneurons [39,61],  
21 cerebellar granule neurons [62], thalamocortical neurons [63,64] and adrenal glomerulosa  
22 cells [65,66]. Whereas different neurotransmitters enhance neuronal excitability through the  
23 inhibition of TASK channels, the inhibition of the channels by circulating angiotensin II in the  
24 glomerulosa cells results in increased production of aldosterone and the consequent  
25 retention of Na<sup>+</sup> and water [66].  
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32 In accordance with the general importance of receptor-mediated inhibition of these  
33 channels, the mechanism of this regulation has been extensively examined in heterologous  
34 expression systems. There has been a long lasting debate regarding the steps leading to the  
35 altered channel activity. Early results revealed the significant role of canonical signalling  
36 pathways (InsP<sub>3</sub>, calcium, and protein kinase C). It has been reported that Gαq can directly  
37 bind to and inhibit TASK-1 [67]. However, a more commonly held view, currently, is that the  
38 activation of phospholipase C (PLC) is required for inhibition of the channels [68-70].  
39 Originally it has been suggested that the breakdown and reduced steady state level of PIP<sub>2</sub>  
40 was responsible for the inhibition [68], particularly, as PIP<sub>2</sub> containing liposomes, or its water  
41 soluble analogues were found to activate TASK-1 in inside-out membrane patches [71].  
42 However, later it has been demonstrated that the depletion of PIP<sub>2</sub> in living cells by  
43 coexpressed lipid phosphatases does not affect TASK-1 activity, and the lipid end product  
44 diacylglycerol (DAG) of PLC enzyme mediates the effect [72]. In addition to the PLC pathway  
45 of regulation, other mechanisms were also reported to inhibit TASK-1 in pulmonary artery  
46 smooth muscle cells. E.g. Rho-kinase inhibits the channel by phosphorylating threonine 393  
47 of TASK-1 in response to endothelin receptor activation [73], and Src tyrosine kinase also  
48 crucially controls TASK-1 channel activity [74]. These mechanisms clearly require further  
49 investigation and they indicate that parallel cell type specific signalling pathways may  
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3 regulate TASK-1 activity in a complex manner as is seen for Gαq mediated regulation of  
4 other K channels.  
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7 TASK-1 plays a key role in the sensing of hypoxic stimuli. Inhibition of TASK-1 by  
8 hypoxia has been extensively studied in dedicated chemosensory (glomus type I) cells  
9 [41,75] (for review see [76]) and the importance of hypoxia related regulation was also clearly  
10 demonstrated in pulmonary resistance vessels [52,56] (for review see [77]). Even before the  
11 discovery of TASK, inhibition of a leak potassium current was known to be a major factor  
12 leading to depolarisation in glomus cells of the carotid body. Later, these leak conductance  
13 were identified as TASK-1/TASK-3 heterodimers and TASK-1 homodimers, the latter being  
14 more sensitive to hypoxia [40,41]. The effects of hypoxia on channel activity are still not well  
15 defined. Reduced pO<sub>2</sub> related to the mitochondrial respiration and the contribution of the  
16 cytoplasmic ATP concentration, AMP kinase, H<sub>2</sub>S and CO were raised. The theory of indirect  
17 regulation is supported by the observed rapid rundown of channel activity during excised  
18 patch experiments following the removal of TASK channels from their natural environment.  
19 The regulatory elements may be active in particular oxygen sensitive tissues (glomus cells,  
20 pulmonary arterioles, and neuroepithelial bodies), while their absence may explain the lack of  
21 response to hypoxia elsewhere and the failure to reproduce this type of regulation in  
22 heterologous expression systems.  
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32 Volatile anaesthetics activate most K<sub>2</sub>P channels and TASK-1 is also a target of  
33 halothane, isoflurane and sevoflurane [78,79] (Figure 5). The activation of TASK-1 in  
34 motoneurons contributes to the immobilizing effects of inhalational anaesthetics [80]. Local  
35 anaesthetics, e.g. lidocaine and bupivacaine non-specifically inhibit TASK-1 at relatively high  
36 concentration [81]. Anandamide and methanandamide are nonselective blockers of TASK-1  
37 and TASK-3 [39,82], however, it remains to be established whether TASK channels mediate  
38 cannabinoid receptor-independent effects of these endocannabinoids. The respiratory  
39 stimulants, doxapram, PKTHPP and A1899 inhibit TASK-1/TASK-3 channels and may act in  
40 the carotid body [40,83,84]; these are potential therapeutic agents in sleep apnea, and it has  
41 been known for decades that doxapram can reverse human respiratory depression induced  
42 by morphine. Another TASK-1/TASK-3 inhibitor A293, which has a slight preference for  
43 TASK-1, was used to demonstrate TASK expression in rat cardiac myocytes [85]. Later  
44 TASK-1/TASK-3 channels were also detected in human atrial cardiac myocytes [42,86].  
45 Upregulation of TASK-1 was reported in patients with chronic atrial fibrillation [87,88].  
46 Interestingly, loss of function mutations in TASK-1 were also found to be associated with  
47 atrial fibrillation [89].  
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57 Inactivating mutations of TASK-1 have also been demonstrated to cause pulmonary  
58 arterial hypertension (PAH) in patients [90], raising the question whether a drug used for  
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3 chronic inhibition of TASK channel in atrial fibrillation would cause pulmonary hypertension  
4 as a side effect and alternatively, whether a TASK activator for the treatment of PAH could  
5 result in atrial fibrillation. Furthermore, TASK-1 is also highly expressed in human adrenal  
6 glomerulosa cells together with the inwardly rectifying K<sup>+</sup> channel Kir3.4 encoded by the  
7 KCNJ5 gene [91,92]. Although the mutations of KCNJ5, resulting in increased Na<sup>+</sup>  
8 conductance are responsible for the clinical cases of primary hyperaldosteronism [93],  
9 pharmacological modulation of TASK-1 may also influence aldosterone production and the  
10 salt water balance. This idea is also supported by the primary hyperaldosteronism evoked by  
11 TASK-1 gene knock-out in rodent models [66,94], and by the association of human TASK-1  
12 (KCNK3) variants with hypertension and high plasma aldosterone levels [95]. In addition,  
13 knock-out mice lacking TASK-1 channels are characterized by impaired carotid body  
14 chemoreceptor function [96]. Pharmacological interventions and therapeutic modalities have  
15 to differentially target these overlapping and important physiological functions of TASK-1 to  
16 avoid adverse effects.  
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## 25 **Figure 5**

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

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29 It is important to realise that the pulmonary circulation differs from systemic circulation  
30 with regards to vasoregulation under hypoxemia, blood pressure regulation and anatomy. i)  
31 in contrast to the systemic circulation, hypoxemia leads to vasoconstriction of small  
32 resistance arteries in the pulmonary circulation. This physiological response is called as  
33 hypoxic pulmonary vasoconstriction and responsible for optimizing the matching of perfusion  
34 and ventilation and preventing arterial hypoxemia [97,98]. ii) The pulmonary circulation lacks  
35 of regulation by central nervous control mechanism. iii) Finally, the large, muscular  
36 pulmonary arteries directly merge into small, partially muscular vessels designed for a very  
37 low perfusion resistance. In the systemic circulation, the arterioles usually have a continuous  
38 thick layer of smooth muscle cells that have a high perfusion resistance.  
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46 Ion channels play a central role for the regulation of the pulmonary vascular tone and  
47 for mediating the effect of physical and chemical stimuli. They can be considered the  
48 executive limb of the response. However, the distribution of the ion channels in the  
49 pulmonary circulation differs from systemic circulation. It is often questioned whether the K<sup>+</sup>  
50 channels are active at sufficiently negative potentials to set the resting membrane potential of  
51 pulmonary artery smooth muscle cells (PASMC) and which K<sup>+</sup> channels could regulate  
52 pulmonary vascular tone [52,54,56,99-104]. Effective modulation of membrane potential by  
53 K<sup>+</sup> channels has been shown in rat PASMC: overexpression of voltage-gated K<sup>+</sup> channels  
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3 (Kv1.5) led to significant hyperpolarisation *in vitro* and reduction of pulmonary vascular  
4 resistance in hypoxia-induced pulmonary hypertension in this species *in vivo* [105,106].  
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7 Recent evidence supports the role of TASK-1 in controlling resting membrane  
8 potential in PASMCs. Its voltage-independent gating makes it a good candidate for the  
9 maintenance of resting membrane potential in cells, where resting membrane potential has  
10 to be kept low in order to keep calcium influx through voltage-gated calcium channels  
11 negligible. TASK-1 is expressed in rabbit [52], mouse [100], rat [54,101] and human [56]  
12 PASMCs and the non-inactivating K<sup>+</sup> current (I<sub>KN</sub>), proposed to be mediated by TASK-1,  
13 shows the distinguishing features of the channel [26,37,76,77]. Most importantly, from the  
14 TASK family only TASK-1 is present in human PASMCs making it particularly susceptible in  
15 the human pulmonary circulation [56]. Despite evidence that K<sup>+</sup> channels control resting  
16 membrane potential, investigations during the last decades confirmed significant inter-  
17 species differences in resting potential in PASMCs and thus, highlighted important inter-  
18 species variability in the physiology of pulmonary arteries. While in rat, rabbit or human  
19 PASMC the resting membrane potential is around -50mV or less, mouse PASMCs have a  
20 resting potential closed to -30 mV. Accordingly, the amplitude of the I<sub>KN</sub> is in a similar range  
21 in rat, rabbit or human PASMC. In contrast, I<sub>KN</sub> in mice is hardly detectable and lacks of the  
22 distinguishing features of I<sub>KN</sub> in other species [100]. Furthermore, the resting membrane  
23 potential or I<sub>KN</sub> do not differ between PASMCs obtained from wild-type and TASK-1/3 knock-  
24 out animals, confirming the lack of functional TASK-1 in the pulmonary arteries in mice.  
25 Thus, evidence is accumulating that where the resting membrane potential of PASMC is  
26 depolarized, I<sub>KN</sub> is absent and TASK-1 not required for the normal pulmonary arterial function  
27 [100]. More recent work shows that TASK-1 channels do not have a role in initiating hypoxic  
28 pulmonary hypertension in murine intra-pulmonary arteries [107]. Indeed, in mice, TASK-1  
29 function seems to be replaced by other K<sup>2P</sup> channels. Accordingly, TWIK-2 (KCNK6)  
30 deficient mice spontaneously develop pulmonary hypertension [108]. In contrast, TASK-1 is  
31 functionally expressed in rats and KCNK3 inhibition with A293 was shown to predispose  
32 pulmonary arteries to constrict [109]. Furthermore, TASK-1 expression and function were  
33 reduced in the monocrotaline-induced pulmonary hypertension model in rats. Together,  
34 these findings question the use of mice as a model to investigate human pulmonary vascular  
35 physiology and especially the functional roles of K<sup>+</sup> channels.  
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### 53 ***TASK-1 in the human pulmonary circulation and its relevance for the human*** 54 ***disease***

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56 Mutations, downregulation or inhibition of K<sup>+</sup> channels has been proposed to  
57 contribute to pulmonary vascular remodelling in man, resulting in pulmonary hypertension  
58 [110,111]. Pulmonary hypertension (PH) is defined by a rise of the mean pulmonary artery  
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3 pressure (mPAP) of 25 mm Hg or more due to a progressive increase of pulmonary vascular  
4 resistance to a level where the right ventricle (RV) compensates by RV hypertrophy and fails  
5 when it is unable to fulfill the rise of afterload. Major advances in the understanding of PH  
6 have led to the current classification in which PH diseases are grouped into five categories  
7 according to cause and therapeutic strategy [112]. The diverse and complex mechanisms  
8 underlying the pathogenesis of pulmonary arterial hypertension (PAH) (group 1 PH) include  
9 vasoconstriction, in-situ thrombosis, progressive vascular remodeling of the small pulmonary  
10 arteries (< 500µm), an excess of vasoconstrictors and a parallel deficiency of vasodilating  
11 mediators [113].

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

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34 In 2013, five different mutations were identified in KCNK3 gene (TASK-1) in PAH  
35 patients (Figure 6). Heterozygous KCNK3 mutations were observed in 1.3% sporadic and  
36 3.2% heritable PAH patients. Patch-clamp experiments demonstrated a loss of function in all  
37 five identified mutations [90]. More recently, two additional KCNK3 mutations have been  
38 identified in Spanish cohort of PAH patients. Interestingly, this report described the first case  
39 of PAH occurring in a patient with homozygous KCNK3 mutations associated with aggressive  
40 form of PAH [116]. To date eight different KCNK3 mutations have been described in PAH  
41 patients (Figure 6). Thus, KCNK3 mutations are the first channelopathies known to cause  
42 PAH to date [90].

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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 analog 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

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3 KCNK3, patch-clamp experiments showed a severe decrease of A293- (specific KCNK3  
4 inhibitor) sensitive current function in cultured PASMC from idiopathic PAH patients  
5 compared to controls [109]. These results suggest that KCNK3 loss of function or decreased  
6 expression is a hallmark of PAH [109].  
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### 9 **Figure 6**

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12 Confirming the crucial role of KCNK3 channels in PAH pathogenesis, endothelin-1  
13 (ET-1), a potent vasoconstrictor for vascular remodeling, has been shown to inhibit KCNK3  
14 via Rho kinase [73] and via a protein kinase (PK) C-dependent pathway in human PASMCs  
15 (Figure 5) [117]. Increased expression of endothelin-1 level has been found in pulmonary  
16 arteries of PAH patients [118]. However, KCNK3 function could be inhibited by downstream  
17 signaling of other G-protein-coupled receptors as diacylglycerol homeostasis directly inhibits  
18 KCNK3 [72]. Interestingly, aminorex, fenfluramine or selective serotonin reuptake inhibitors,  
19 drugs associated with an increased risk for the development of PAH act via these pathways  
20 [119]. In addition, the non-receptor tyrosine kinase activity seems to be essential for the  
21 function of TASK-1 since targeted inhibition of c-src by siRNA reduces TASK-1 current in  
22 human PASMCs [74]. The recently reported association of severe PAH with dasatinib, the c-  
23 Src kinase inhibitor used in the treatment of chronic myelogenous leukaemia suggests a  
24 direct and specific effect of s-src inhibition on pulmonary vessels [120]. It is noteworthy, that  
25 in PAH patients the protein expression of the non-receptor tyrosine kinase c-src is  
26 significantly reduced in the lung [121]. These findings demonstrate the key role of TASK-1 in  
27 many different pathways leading to PAH.  
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37 Beyond the direct effects of the TASK-1 inhibition on the vasoreactivity in the  
38 pulmonary circulation, the role of KCNK3 channel in the proliferation/apoptosis balance of  
39 human PASMC remained unknown. Recent investigations demonstrated that *in vivo* chronic  
40 KCNK3 inhibition in rats induced an exaggerated proliferation of PAECs, PASMCs, and  
41 adventitial fibroblasts, which could initiate or promote the development of pulmonary  
42 hypertension [109]. In addition, the membrane potential of PASMC from KCNK3 deficient  
43 rats, generated by using CRISPR-Cas9 technology, are significantly depolarized and the  
44 mutation induced distal neomuscularization, abnormal pulmonary arteries vasoreactivity and  
45 elevated mean right ventricular systolic pressures, confirming that KCNK3 loss of function is  
46 a key event in PAH pathogenesis [122].  
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### 56 **Conclusion and future directions**

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3 Both preclinical and clinical studies strongly support TASK-1 channels as important players  
4 in the pathology of pulmonary vascular diseases. As TASK-1 channels regulate resting  
5 membrane potential in human PASMC and consequently low pulmonary vascular tone, in  
6 order to achieve maximum vasodilation under pathophysiological conditions, restoring TASK-  
7 1 channel function channel is a viable therapeutic approach. Although, TASK-1 has an  
8 accessible cell surface location and considerable tissue-defined distribution, TASK-1 remains  
9 underexploited as a target in drug discovery. This may be due to a number of factors. There  
10 is a limitation of primary human lung tissue and the drug delivery has to be tissue specific to  
11 avoid unpredictable side effects even for specific activators. High-throughput screening  
12 methods for ion channel targets lack temporal resolution over a physiologically relevant  
13 range and manual patch-clamping is time consuming. In addition, channel modifiers often  
14 need to bind to relatively inaccessible sites within the channel pore or to accessory or  
15 regulatory domains. In silico modeling and advances in structural biology techniques to  
16 crystallize channel proteins in complex with accessory subunits may reveal key interaction  
17 sites and interfaces for drug design. Indeed, modulating channel behavior, rather than  
18 directly targeting the ion-conducting subunit may ultimately be a more fruitful approach. In  
19 this context, the combination therapy for cystic fibrosis using lumacaftor and ivacaftor might  
20 suggest a novel therapeutic direction. In the case of cystic fibrosis, Lumacaftor increases  
21 expression of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl<sup>-</sup> channel  
22 at the cell surface and ivacaftor increases its open probability. The need for novel, effective  
23 ion channel modulators exists but now the challenge is to match therapeutic strategy with  
24 innovative design.  
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## Figure legends

### 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 colored blue-to-red from the N to the C terminus, and the other subunit is gray.  $K^+$  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 colored green. b. An orthogonal view of the channel from the side. c. Secondary structure of K2P colored according to a. Dashed lines indicate disordered regions. (with permission from [124]).

### 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 (adapted from [123]). b. Ion-flux gating of K2P channels. c. Rectification coefficients (currents at +100mV/-100mV) subsequent to a depolarizing pulse to +100 mV (from a holding voltage of -100 mV) are shown for the indicated K2P channels (adapted from [13]).

### 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  $\pm$  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  $\pm$  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

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relationship: 
$$I_{K^+} = \bar{P}_{K^+} \cdot \left( \frac{[K^+]_{out}}{K_{0.5} + [K^+]_{out}} \right) \cdot \left( \frac{V_m F^2}{RT} \right) \cdot \frac{[K^+]_{in} - [K^+]_{out} \cdot e^{-V_m F/RT}}{1 - e^{-V_m F/RT}}$$
 where  $I_{K^+}$  is the potassium current,  $\bar{P}_{K^+}$  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  $K^+$ . (with permission from [125]).

#### 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 depolarization and subsequent  $Ca^{2+}$  entry through nifedipine-sensitive L-type  $Ca^{2+}$  channels.

#### Figure 5. TASK-1 in pulmonary arterial smooth muscle cells.

**a.** Regulation of TASK-1 in pulmonary arterial smooth muscle cells. Em: membrane potential; PIP2: phosphatidylinositol-4,5-bisphosphate; IP3: inositol-1,4,5-trisphosphate; 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;  $Ca^{2+}$ : Calcium,  $K^+$ : potassium. **b.** Topologic analysis of the human KCNK3/TASK-1 channel. Positions indicating the mutations identified by Ma L et al., [90], and Tejedor N et al., [116].

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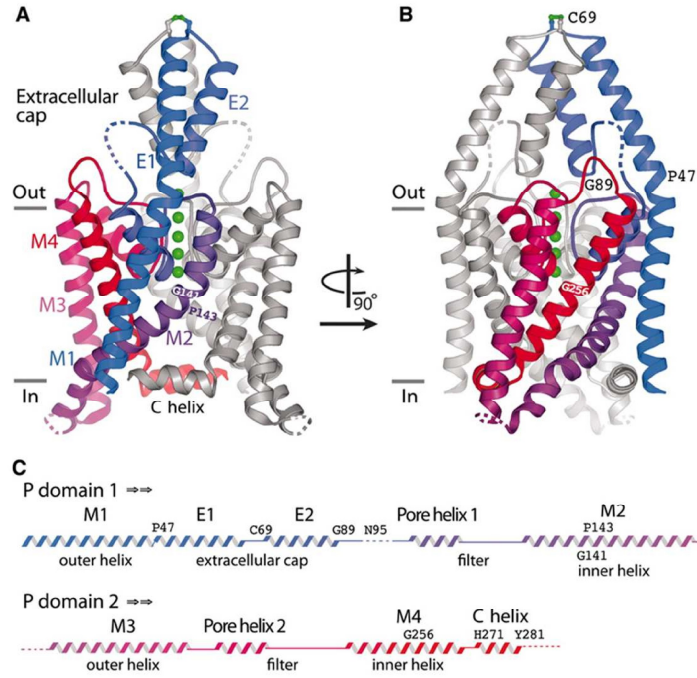


Figure 1

279x210mm (96 x 96 DPI)

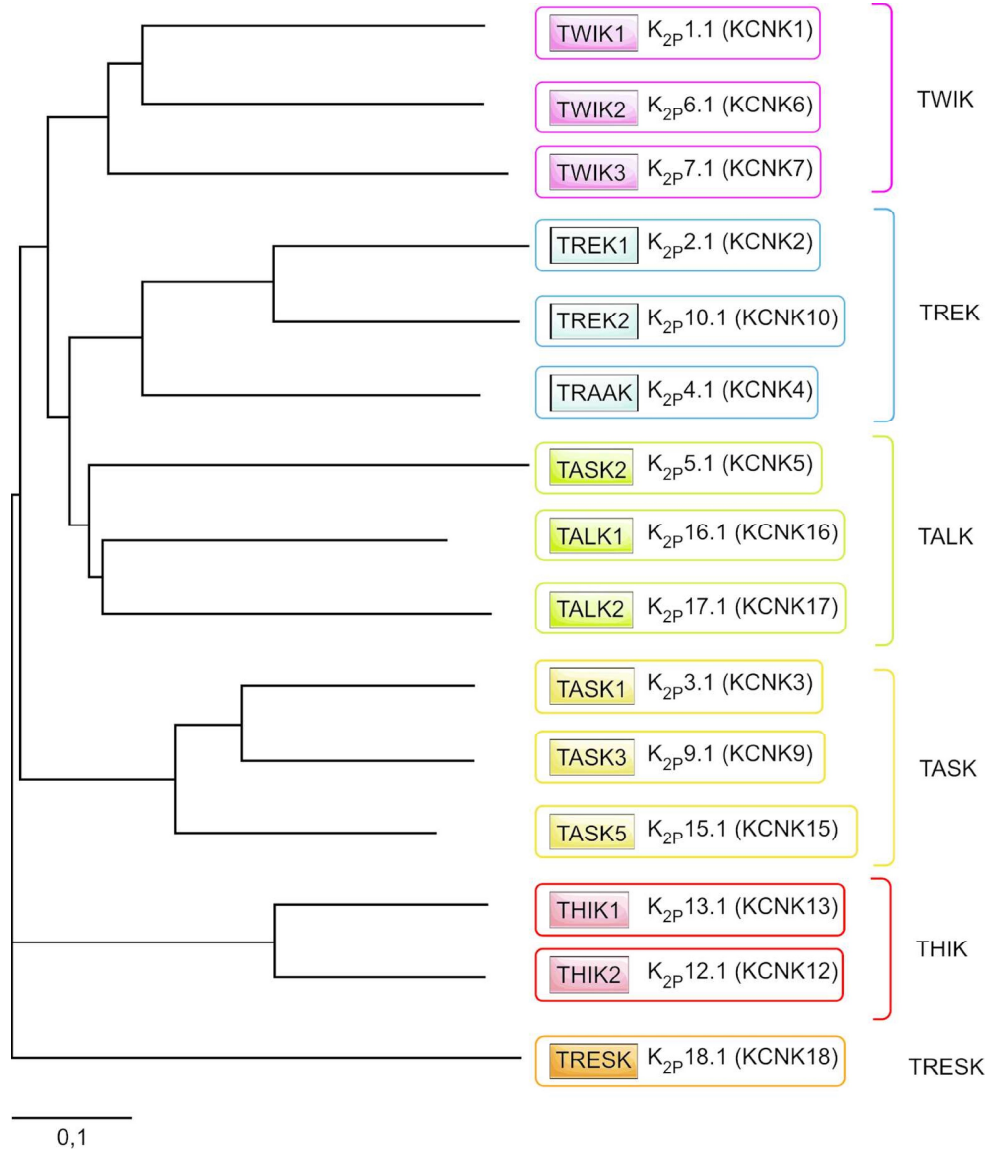


Figure 2a

120x141mm (300 x 300 DPI)

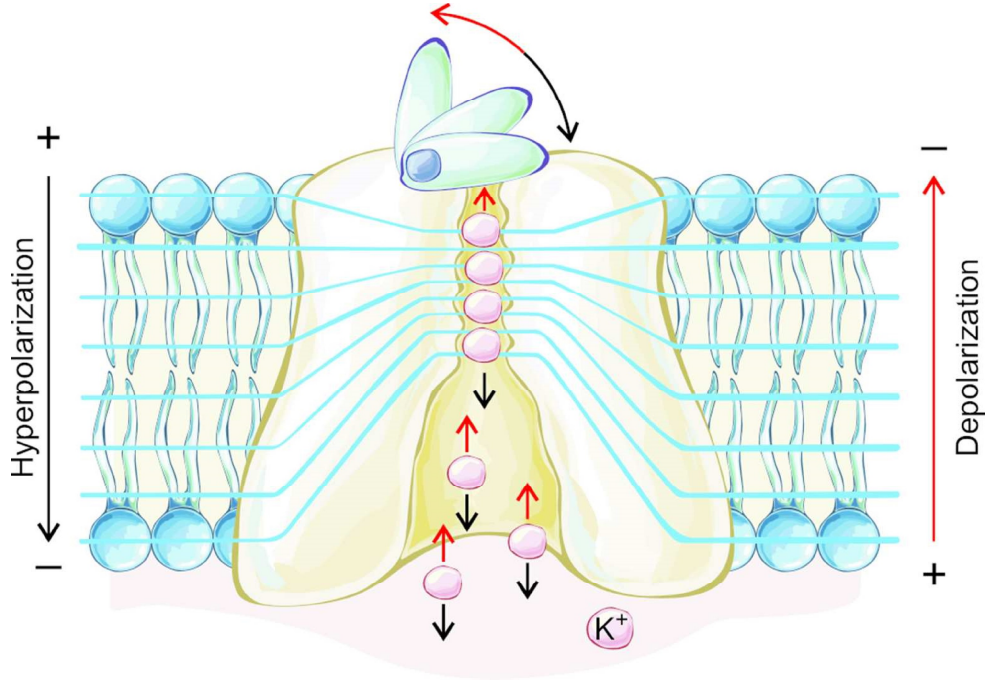


Figure 2b

88x61mm (300 x 300 DPI)

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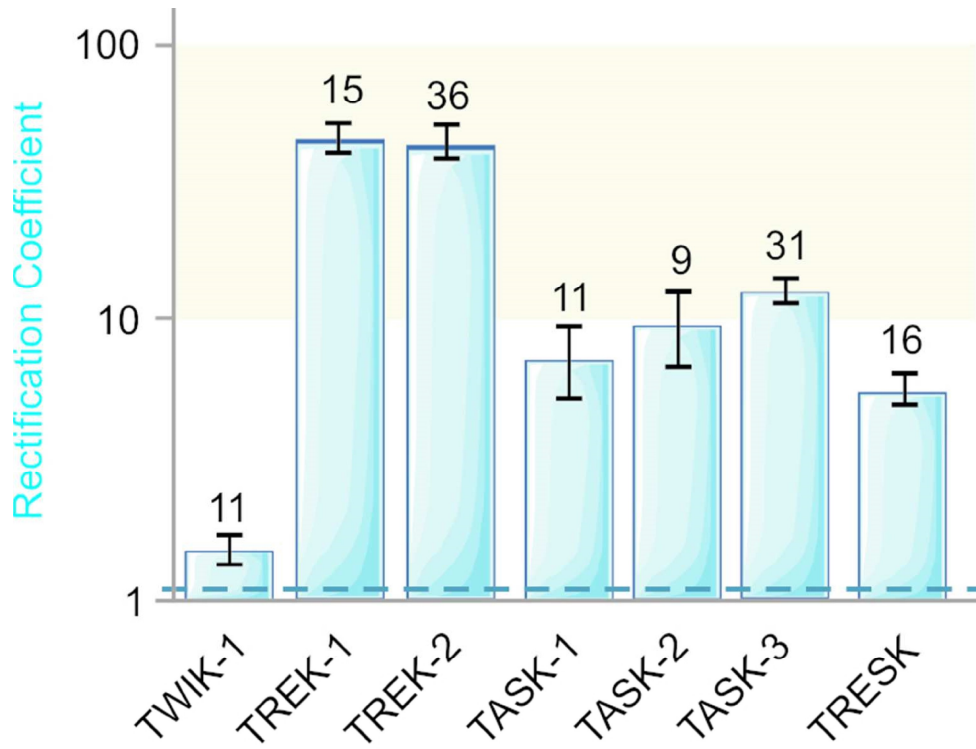


Figure 2c

68x52mm (300 x 300 DPI)

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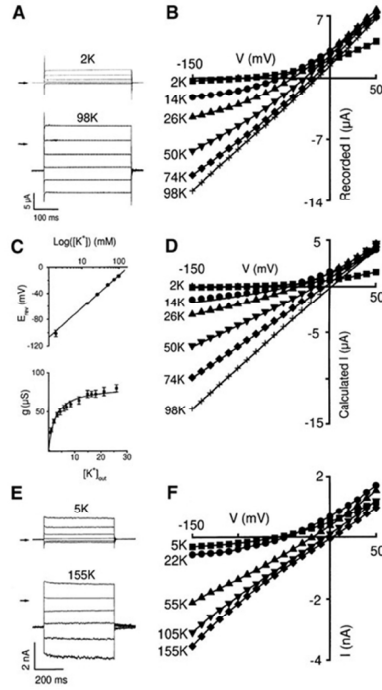


Figure 3

279x210mm (96 x 96 DPI)



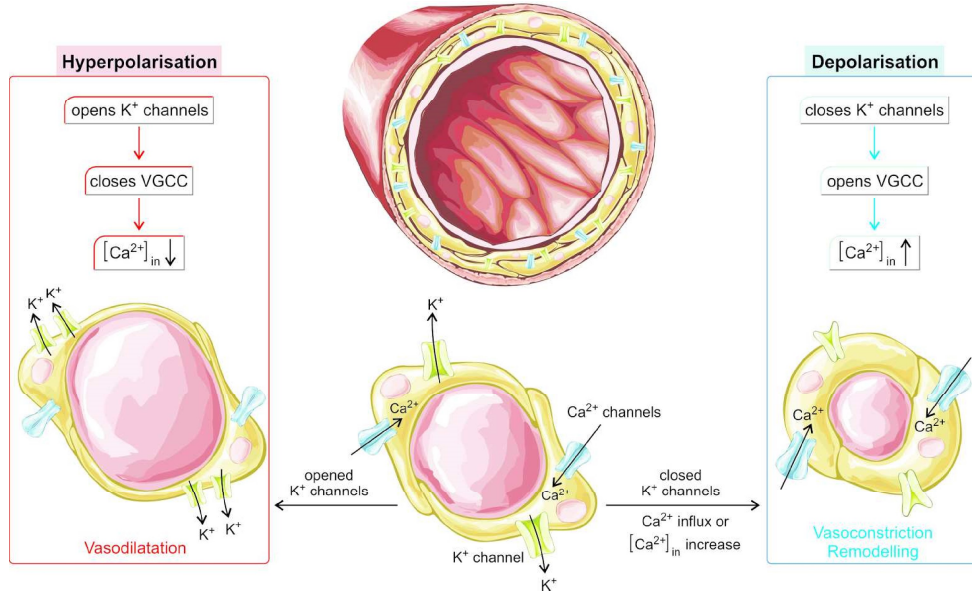


Figure 4

187x113mm (300 x 300 DPI)

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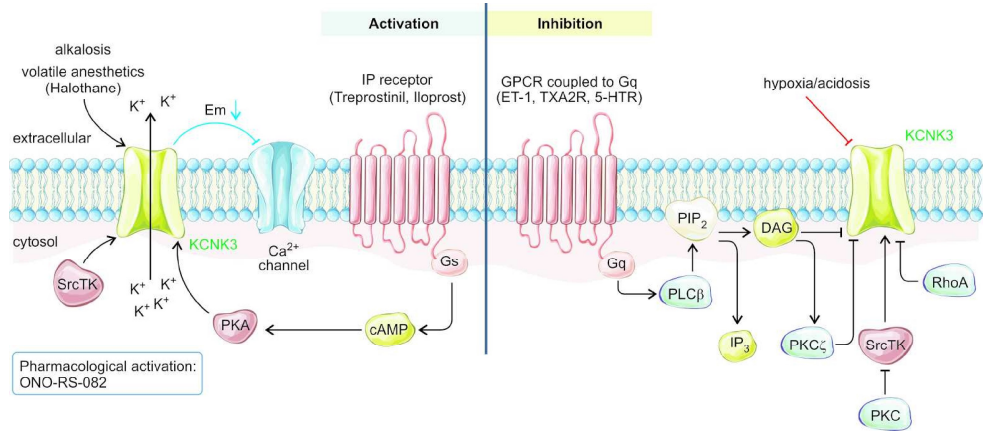


Figure 5a

188x82mm (300 x 300 DPI)

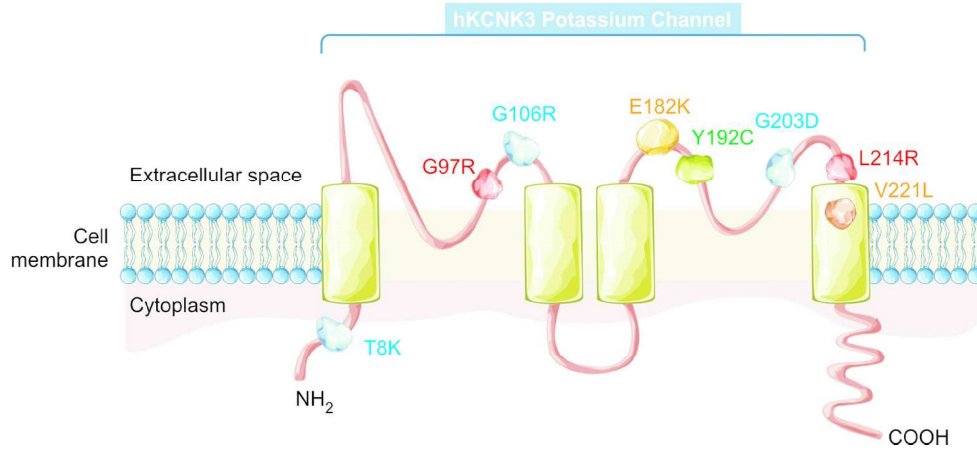


Figure 5b

134x60mm (300 x 300 DPI)

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