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REVIEW

Molecular physiology of oxygen-sensitive potassium channels

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ABSTRACT: Physiological adaptation to acute hypoxia involves oxygen-sensing by a variety of specialized cells including carotid body type I cells, pulmonary neuroepithelial body cells, pulmonary artery myocytes and foetal adrenomedullary chromaffin cells.

Hypoxia induces depolarization by closing a specific set of potassium channels and triggers cellular responses. Molecular biology strategies have recently allowed the identification of the K^+ channel subunits expressed in these specialized cells.

Several voltage-gated K^+ channel subunits comprising six transmembrane segments and a single pore domain (Kv1.2, Kv1.5, Kv2.1, Kv3.1, Kv3.3, Kv4.2 and Kv9.3) are reversibly blocked by hypoxia when expressed in heterologous expression systems. Additionally, the background K^+ channel subunit TASK-1, which comprises four transmembrane segments and two pore domains, is also involved in both oxygen- and acid-sensing in peripheral chemoreceptors.

Progress is currently being made to identify the oxygen sensors. Regulatory β subunits may play an important role in the modulation of Kv channel subunits by oxygen.

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A rapid response to environmental hypoxia is a reflex increase in the respiration rate. This reflex is initiated in the carotid bodies located at the bifurcation of the carotid arteries [1, 2]. Upon a decrease in arterial oxygen tension (P_{a,O_2}), the chemoreceptor type I carotid body cells release neurotransmitters, which activate afferent sensory fibres of the sinus nerve stimulating the brainstem respiratory centres and provoking a reflex increase in ventilation. Similarly, neuroepithelial body cells, which are innervated clusters of amine and peptide containing cells located within the airway mucosa, are transducers of hypoxic stimuli and function as airway chemoreceptors in the regulation of the respiration [3–5]. In the perinatal period, before the establishment of sympathetic innervation, hypoxia additionally triggers catecholamine release from adrenomedullary chromaffin cells [6]. This "non-neurogenic" mechanism is essential for the newborns ability to survive hypoxic stress and modulates respiratory as well as cardiovascular and metabolic responses. Besides the release of neurotransmitters and the stimulation of respiration, hypoxia also has a profound adaptive effect on the pulmonary circulation [7, 8]. Hypoxia-induced vasoconstricion of resistance pulmonary artery smooth muscle (PASM) leads to a redistribution of the nonoxygenated blood towards better ventilated regions of the lung. The low oxygen tension environment of the foetus also contributes to the high foetal pulmonary vascular resistance. The blood ejected from the right ventricle of the foetus bypasses the pulmonary circulation through the opened ductus arteriosus [9, 10]. At birth, the increase in oxygen tension produces a rapid fall in pulmonary artery resistance while it provokes the closing of the ductus arteriosus. Although hypoxic pulmonary artery vasoconstriction fulfills an essential physiological function in matching ventilation and perfusion, it also contributes to the development of pulmonary artery hypertension in patients with chronic obstructive lung diseases and people living at high altitude [7].

These specialized cells share in common a mechanism which transduces hypoxic stimuli into a cellular response: the closing of oxygen-sensitive potassium channels [1, 2, 7, 8]. Hypoxia depolarizes the oxygen-sensitive cells, increases excitability, provokes the opening of voltage-gated Ca^{2+} channels, increases intracellular Ca^{2+}, and triggers cellular responses including neurotransmitter release as well as myocyte contraction.

Recent progress has been made in identifying the molecular components of the oxygen-sensitive K^+ channels. First, the molecular diversity of the mammalian K^+ channel α and β subunits will be described. Second, the expression of the oxygen-sensitive K^+ channels subunits in carotid bodies, neuroepithelial bodies, PASM, as well as in the clonal cell lines PC12 and H-146 will be detailed. Finally, the molecular mechanisms involved in the regulation of these K^+ channel subunits by oxygen will be discussed.

Molecular diversity of potassium channels α subunits

K^+ channels are multimers of α subunits which form the ionic pore. In mammals, >70 α subunits have been cloned forming the largest family of ion channels [11] (fig. 1). K^+ channels can be divided into three distinct structural classes according to their membrane topology. The largest group comprises subunits with six transmembrane segments and a single pore domain (P). The P-domain is a conserved region between all the K^+ channels from bacteria to human, and determines K^+ ion selectivity [12]. Both amino and carboxy terminals are intracellular. The six transmembrane segment and one P-domain channels comprise the voltage-gated (Kv) and the calcium-activated (SK, Slo) K^+ channels (fig. 1). Functional channels are tetramers of either identical subunits (homomultimers) or different subunits (heteromultimers) (fig. 2). The assembly domain T1 is part of the cytosolic amino terminus [14, 15]. Heteromultimers are functionally different from homomultimers and contribute to increase the level of complexity [11]. Some Kv channels (Kv1.4, Kv3.3 and Kv3.4) are characterized by a fast voltage-dependent inactivation. The cytoplasmic amino terminus of the channel can be visualized as a positively charged ball that provokes fast closing of the channel by occluding the pore once it is opened (fig. 2).

The second structural family comprises K^+ channels with two transmembrane segments and one P-domain (fig. 1). Again, four subunits associate to form a functional channel. The inward rectifiers (IRK), the adenosine triphosphate (ATP)-dependent K^+ channels (KATP) and the G-protein regulated K^+ channels (GIRK) belong to this structural class [11, 16].

The last structural family is made of K^+ channels with four transmembrane segments and two P-domains [17] (fig. 1). These subunits have been proposed to dimerize. These genes encode background K^+ channels which are sensitive to stretch, fatty acids, anaesthetics, internal and external pH and are either positively or negatively regulated by G protein-coupled membrane receptors [17].

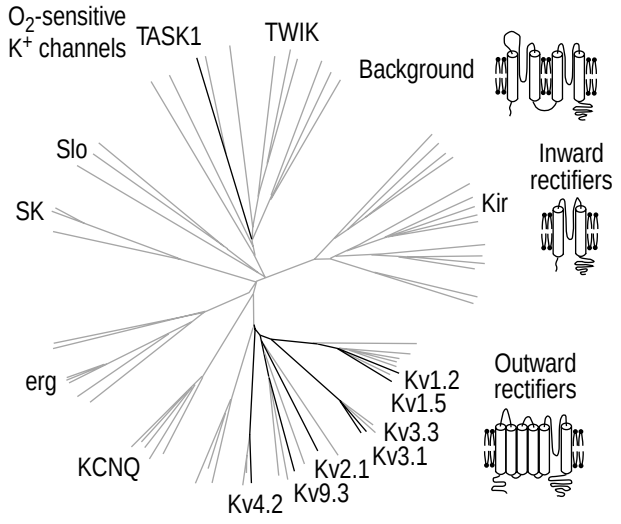

Fig. 1. – Phylogenetic tree of the potassium (K^+) channel α subunits. Subunits proposed to be involved in oxygen (O_2)-sensing are indicated in black.

Molecular diversity of potassium channels β subunits

Regulatory β subunits modulate the biosynthesis and the gating of K^+ channels α subunits. Three main regulatory Kvβ subunits have been identified (Kv β1-3) [18]. Kvβ subunits interact with the amino terminus of the α subunits [14, 19] (fig. 2). Four β subunits interact with four α subunits to form a functional channel. Again, heteromultimeric association of different β subunits contribute to increase the complexity of Kv channels. The amino terminus of Kvβ1 and Kvβ3 are similar to the inactivation ball of Kv1.4, Kv3.3 and Kv3.4 α subunits. Co-expression of Kvβ1 or Kvβ3 subunits with a Kv1.X subunit lacking an inactivation ball yields a fast inactivating K^+ channel [18]. The 3D structure of Kvβ2 has recently been solved by X-ray crystallography [19]. Each subunit is an oxidoreductase enzyme with a nicotinamide cofactor in its active site. The α-β complex is oriented with four T1 domains, forming a ring facing the transmembrane pore and four β subunits facing the cytoplasm [14] (fig. 2). The transmembrane pore communicates with the cytoplasm through lateral, negatively charged openings above the T1(4) β (4) complex (hanging gondola) [13, 19] (fig. 2). The inactivation peptides of voltage-dependent K^+ channels (the positively charged amino terminus of some Kvα and Kvβ) reach their site of action by entering these openings (fig. 2). β subunits comprising two transmembrane segments modulate the activity of the calcium-dependent Slo channels.

Subunit composition of the oxygen-sensitive potassium channels in type I carotid body and pheochromocytoma PC-12 cells

The primary sensory cells of the carotid body respond to hypoxia and acidosis with a depolarization initiating electrical activity, calcium entry and neurosecretion [2, 20–25]. A key ionic current involved in mediating these responses in rat type I cells is an oxygen- and acid-sensitive background K^+ current [26, 20, 21]. This current displays a baseline activity with no voltage and time-dependency and shares all the biophysical and pharmacological properties of the cloned K^+ channel TASK-1, a member of the four transmembrane segments and two P-domains family [26–30] (fig. 1). TASK-1 is insensitive to cesium, tetraethylammonium and 4-aminopyridine, although it is blocked by barium, quinine, quinidine and zinc. TASK-1 is opened by inhalational general anaesthetics including halothane, although it is blocked by local anaesthetics such as bupivacaine [28, 29]. A key feature of TASK-1 is its high sensitivity to external pH with half-inhibition at pH 7.3 [27]. *In situ* hybridization and reverse transcriptase-polymer chain reaction (RT-PCR) analysis (unpublished data) have shown

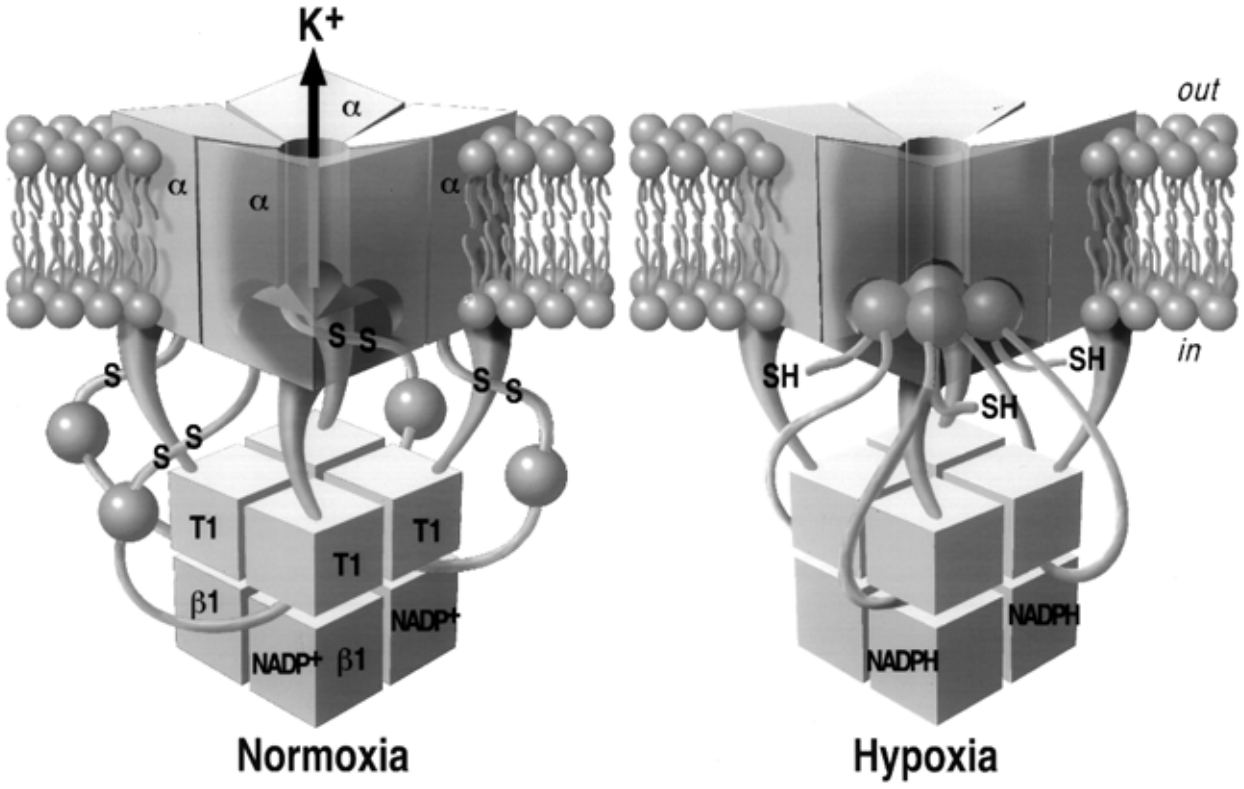

Fig. 2. – Illustration of an α-β potassium (K^+) channel complex A tetramer of α subunits forms the ionic pore. β subunits interact with the assembly domains T1 in the cytosol. A positively charged amino terminal ball domain of the α subunit (and possibly for the β subunit) underlies fast inactivation. The reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex is shown to associate with this K^+ channel complex in neuroepithelial body cells. Reactive oxygen intermediate produced by the NADPH oxidase, modulate the inactivation process by oxidizing specific cysteine residues in the amino terminus, forming disulphide bridges with other cyteines located in the channel and thus immobilizing the inactivation balls. Adapted from [3, 13].

that TASK-1 messenger ribonucleic acid (mRNA) is abundant in type I carotid body cells [26]. Due to its baseline activity, TASK-1 sets the resting membrane potential and thus controls cell excitability. In cell-attached patches of rat type I carotid body cells, hypoxia closes TASK-1-like channels, while it has no effect in the inside-out patch configuration [26]. The loss of oxygen sensitivity upon excision suggests that some cytosolic messenger or cofactor may be required to confer or maintain oxygen sensitivity of TASK-1 [26]. When expressed in transfected cells or in Xenopus oocytes, TASK-1 is not significantly altered by hypoxia (unpublished data). Recently, the endogenous cannabinoid anandamide (the amide of AA), a product of phospholipase D, has been shown to be a direct and selective blocker of TASK-1 [31]. It will be interesting to assay the functional role of anandamide or a related molecule in the hypoxic inhibition of TASK-1 in carotid body cells.

Hypoxia initiates the neurosecretory response of the carotid body by inhibiting several types of potassium channels [20, 23, 25]. Besides the background TASK current in rat type I cells, a transient outward K^+ current is also reversibly inhibited by hypoxia in rabbit type I cells [25]. A dominant negative strategy using adenovirus gene transfer has been recently used to elucidate the functional role of Shal (Kv4.X) subunits in the hypoxic depolarization of rabbit type I carotid body cells [25] (fig. 1). The Kv1.X dominant negative has no effect, although the Kv4.X dominant negative completely suppresses the transient outward current, depolarizes cells and abolishes the hypoxia-induced depolarization [25]. When expressed in HEK293 cells, Kv4.2 is not sensitive to hypoxia or redox stimulation [32]. However, co-expression of Kv4.2 with Kvβ1.2 forms a K^+ channel which is reversibly inhibited by hypoxia [32]. This work suggests that in rabbit type I carotid body chemoreceptor cells, a Shal subunit in association with a Kvβ subunit may encode the oxygen-sensitive transient outward K^+ channel [25] (fig. 2). The hypoxic inhibition of Kv4.2/Kvβ1.2 was similarly observed in the outside-out excised patch configuration suggesting that acute hypoxia is acting through a membrane-delimited pathway [32]. These data imply that either Kv4.2/Kvβ1.2 proteins are intrinsically oxygen-sensitive or, alternatively, there is a closely associated but distinct oxygen-sensing element that is endogeneously expressed in the membrane of the host cell. Each β subunit is an oxidoreductase enzyme with a nicotinamide cofactor in its active site [18]. The cofactor can be either in its reduced, nicotinamide adenosine dinucleotide phosphate (NADPH) or oxidized ($NADP^+$)form. The gating conformational state of the K^+ channel could be coupled to the β subunit active site [18] (fig. 2). The β subunit might

thus function as an intracellular redox-sensing device modulating the activity of Kv4.2 channels. A haemoproteic oxygen sensor might additionally be involved (at least in HEK cells) as carbon monoxide (CO) reverts hypoxic inhibition of Kv4.2/Kvβ1.2 [32].

Both carotid body type I cells and pheochromocytoma PC12 cells originate from neural crest and synthesize dopamine as their major neurotransmitter response in low partial oxygen pressure (PO_2) conditions [33, 34]. Hypoxia induces depolarization of PC12 cells by inhibiting voltage-gated slowly inactivating K^+ channels. RT-PCR analysis revealed that Kv1.2, Kv1.3 together with Kv2.1, Kv3.1 and Kv3.2 channel genes are expressed in PC12 cells [33, 34] (fig. 1). The expression of the Shaker α subunit Kv1.2 but none of the other K^+ channel genes, increases in PC12 cells exposed to prolonged hypoxia (18 h) [33]. Moreover, cells maintained in chronic hypoxia become more responsive to a subsequent acute hypoxia when compared to cells maintained in normoxia [33]. Charybdotoxin, a blocker of Kv1.2, inhibits the oxygen-sensitive K^+ current in PC12 cells [34]. Moreover, intracellular administration of an anti-Kv1.2 antibody completely abolishes this current, while an anti-Kv2.1 antibody has no effect [34]. Finally, hypoxia inhibits Kv1.2 when expressed in Xenopus oocytes, although it fails to affect Kv2.1 [34]. These results suggest that Kv1.2, unlike Kv2.1, encodes the oxygen-sensitive voltage-gated slowly inactivating K^+ channel in PC12 cells [34–33].

Subunit composition of the oxygen-sensitive K^+ channels in neuroepithelial body and in small cell lung carcinoma H-146 cells

Pulmonary neuroepithelial bodies are composed of innervated clusters of amine and peptide containing cells widely distributed throughout the airway mucosa [3, 4, 5]. These cells are functionally similar to the type I carotid body cells and behave as lung chemoreceptors. Hypoxic inhibition of K^+ channels induces cell depolarization and neurotransmitter release [5].

Neuroepithelial body cells express the oxygen-binding protein NADPH oxidase on their plasma membrane [5]. Hypoxia affects the function of the oxidase *via* a decrease in the availability of substrate oxygen, resulting in reduced production of oxygen-reactive intermediates including H_2O_2. Kv1.4, Kv3.3 and Kv3.4 lose their fast inactivation upon external application of H_2O_2 and upon excision of the patches in the inside-out configuration [3, 35, 36, 37] (fig. 2). This effect is reversible when a reducing agent (reduced glutathione or dithiothreitol) is added to the bath or when cramming the patch in the reducing intracellular medium of the cell. Oxidation of a cysteine in the amino terminus of these channels is responsible for the impairment of inactivation and consequent increase in current amplitude [36]. This regulation is probably based on a fast and reversible formation of disulphide bridges between the inactivating ball domain and another part of the protein [36] (fig. 2). The H_2O_2-sensitive channel Kv3.3a is co-expressed with the membrane components of the NADPH oxidase ($gp91^{phox}$ and $p22^{phox}$) in neuroepithelial body cells of foetal rabbit and neonatal human lungs [3]. Exposure of neuroepithelial body cells to hydrogen peroxide results in an increase in K^+ channel amplitude [3]. The critical role of NADPH oxidase as the oxygen sensor of neuroepithelial body cells was demonstrated in $gp91^{phox}$ knock-out mice [4]. Hypoxia fails to inhibit K^+ channels in the oxidase-deficient mice [4]. NADPH oxidase is, however, not a universal oxygen sensor, as hypoxic inhibition of K^+ channels in PASM cells is unaltered in oxidase knock-out animals [38].

The small-cell lung carcinoma cell line H-146 derives from the same precursor pool as neuroepithelial body cells [39]. H-146 cells express oxygen-sensitive K^+ channels. These channels are resistant to 4-aminopyridine but are completely blocked by quinidine [39]. This pharmacology is similar to that of TASK-1 (fig. 1). RT-PCR analysis revealed, as described for carotid body type I cells, the abundant expression of TASK-1 in H-146 cells [26, 39]. However, the oxygen-sensitive K^+ current in H-146 cells is insensitive to external acidosis, the hallmark of TASK-1 [27, 39]. Possible heteromultimeric association of TASK-1 with another acid-insensitive two P-domain K^+ channel subunit could thus be involved. However, heteromultimeric association of four transmembrane segments K^+ channel subunits remains to be demonstrated.

Subunit composition of the oxygen-sensitive Kv channels in pulmonary artery myocytes

Hypoxic pulmonary artery vasoconstriction is at least partly due to the closing of voltage-gated charybdotoxin-resistant and 4-aminopyridine-sensitive Kv channels in PASM cells [7, 8]. Kv1.2, Kv2.1 and Kv9.3 α subunits belonging to the voltage-gated Shaker and Shab subfamilies have been identified in rat PA rings using a degenerate polymerase chain reaction (PCR) strategy [40] (fig. 1). A weaker expression of Kv1.3 was additionally found in cultured PASM cells [40]. Kv1.2 and Kv1.3 were excluded as candidate oxygen-sensitive K^+ channel components as they are both blocked by charybdotoxin. Kv2.1 is a slowly-inactivating charybdotoxin-resistant and 4-aminopyridine-sensitive voltage-gated K^+ channel [40, 41]. As an homomultimer, Kv9.3 does not express a functional current [40]. However, Kv9.3 forms a heteromultimer in association with Kv2.1. Importantly, Kv9.3 shifts the activation threshold of Kv2.1 from ~-20 mV to -50 mV, which is in the range of the resting membrane potential of PA myocytes [40]. The Kv2.1/Kv9.3 heteromultimer might thus contribute to setting up the resting membrane potential of these cells. At the single channel level, Kv9.3 increases the conductance of Kv2.1 from 8 to 14 pS (physiological K^+ gradient) [40]. The expression of Kv2.1 was subsequently demonstrated by Western blot analysis in rat and ovine PA myocytes [42–44]. Interestingly, the increase in Kv2.1 channel activity parallels the increase in hypoxic pulmonary vasoconstriction that occurs with

maturation in ovine pulmonary vasculature [42]. Both the Kv2.1 homomultimer and the Kv2.1/Kv9.3 heteromultimer are reversibly inhibited by hypoxia when expressed in COS and L cells [40, 45]. Intracellular administration of an anti-Kv2.1 antibody inhibits whole cell K^+ current and depolarizes rat PASM cells [43]. Anti-Kv2.1 also elevates resting tension and diminishes 4-aminopyridine induced vasoconstriction in membrane-permeabilized rat PA rings [43]. Hypoxic inhibition of Kv2.1/Kv9.3 which is active in the range of the resting membrane potential of PASM cells, may thus contribute significantly to hypoxic pulmonary artery vasoconstriction [43, 40]. Kv2.1 and Kv2.1/Kv9.3 channels are stimulated by intracellular ATP in excised inside-out patches [40]. ATP modulation of Kv channel activity might be the link between mitochondrial respiration and cell electrogenesis. The fall in intracellular ATP concentration during hypoxia might reduce Kv2.1/Kv9.3 current amplitude and thus lead to depolarization and contraction of PASM cells [40]. Moreover, mitochondrial uncouplers as well as deoxyglucose, inhibit PA myocyte Kv currents [46–47].

RT-PCR, Northern blot as well as Western blot analysis confirmed the expression of Kv1.2, Kv2.1 and Kv9.3 in PASM cells and further revealed the presence of Kv1.1, Kv1.4, Kv1.5, Kv1.6 for the α subunits and Kvβ1.1, Kvβ2 and Kvβ3 for the β subunits [44] (fig. 1). However, in the study of PATEL *et al.* [40], Kv1.5 was not detected by PCR using specific primers [40]. Low complementary deoxyribonucleic acid (cDNA) template concentration as well as weak primer efficiency might explain this discrepancy. Nevertheless, it should be kept in mind that Kv1.5 is highly expressed in PA endothelial cells and that cell contamination could lead to an overestimation of Kv1.5 expression in PASM cells [48, 43]. Kv1.2, unlike Kv1.5, is reversibly inhibited by hypoxia when expressed in L cells [45]. However, Kv1.2 is not active in the range of PASM cell resting membrane potential [45]. As previously observed for Kv2.1 and Kv9.3, co-expression of Kv1.2 with Kv1.5 yields a heteromultimeric dendrotoxin-resistant K^+ channel active at relevant physiological potential which is reversibly inhibited by hypoxia [45]. These results suggest that both heteromultimers Kv2.1/Kv9.3 and Kv1.2/Kv1.5 may encode the oxygen-sensitive K^+ channels in PA myocytes [45, 40].

Intracellular administration of anti-Kv1.5 antibody, unlike anti-Kv2.1, neither caused depolarization nor elevated rat basal pulmonary artery tone [43]. It was suggested that Kv2.1 is primarily inhibited by hypoxia and that hypoxic depolarization caused by Kv2.1 block (probably associated to Kv9.3) shifts the membrane potential into a range where Kv1.5 (probably associated to Kv1.2) is active [43]. Hypoxic inhibition of Kv1.2/Kv1.5 may amplify depolarization [43, 45].

Chronic hypoxia causes a reduction in K^+ channel current amplitude and a depolarization of PA myocytes, a steady increase in pulmonary arterial pressure, a decrease in pressor response to acute hypoxia and ultimately a remodelling with hypertrophy and proliferation of the myocytes [49, 50]. Chronic hypoxia downregulates Kv1.1, Kv1.2, Kv1.5, Kv2.1, Kv4.3 and Kv9.3 mRNA and/or protein expression in cultured rat PASM cells without affecting mRNA levels of Kvβ1-3 subunits [50, 51]. Unchanged transcription of β subunits may increase the fraction of α subunits that are associated with β subunits and thus reduce Kv currents by promoting inactivation [50]. These data suggest that down modulation of Kv channels transcription during chronic hypoxia may cause pulmonary vasoconstriction and hypertension [49, 50].

Recently, it was shown that Kv3.1b is expressed in rabbit PASM as well as in PC12 cells [52] (fig. 1). Moreover, Kv3.1b is reversibly inhibited by hypoxia when expressed in L929 cells [52]. Hypoxic inhibition remains in excised patches, suggesting a membrane-delimited mechanism. The role that Kv3.1b plays in hypoxic pulmonary artery vasoconstriction is unclear as the voltage activation threshold is outside the range of membrane potentials encountered in resting rabbit PASM cells [52]. Association with another Kvα or β subunit might shift the activation threshold, as observed in the case of Kv2.1/Kv9.3 and Kv1.2/Kv1.5 [52, 45, 40].

Conclusions and perspectives

Within the last 4 yrs, several oxygen-sensitive K^+ channels subunits have been identified: Kv1.2 (PC12, PA), Kv1.5 (PA), Kv2.1 (PA), Kv3.1 (PA, PC12), Kv3.3 (neuroepithelial body, H-146), Kv4.2 (carotid body), Kv9.3 (PA) and TASK-1 (carotid body H-146) [3, 25, 26, 32, 33, 34, 39, 40, 43, 45, 52]. These channels are open in the presence of oxygen, but reversibly closed by hypoxia. Interestingly, in rabbit and human ductus arteriosus, hypoxia opens, while oxygen reversibly closes voltage-gated K^+ channels leading to depolarization and constriction of the myocytes at birth [9, 10].

Kv1.2, Kv1.5, Kv2.1 and Kv3.1 are expressed in both PA and ductus arteriosus smooth muscle cells [9, 40, 43, 44]. The same α Kv channel subunits may thus be differentially regulated by oxygen depending on the host cell. For instance, hypoxic sensitivity of Kv2.1 is consistently reported in cDNA-transfected L cells, only observed in a subset of transfected COS cells, while absent in mRNA-injected Xenopus oocytes [34, 40, 45]. These results indicate that regulatory proteins differentially expressed in specialized cells such as PA or ductus arteriosus myocytes, may be required to confer or maintain differential oxygen sensitivity. In neuroepithelial body cells, it is now clear that NADPH oxidase is the oxygen sensor that regulates Kv3.3 function by a redox-modulation of its fast amino terminal-dependent inactivation [4]. In carotid body cells, the Kvβ1.2 protein, which is an active oxidoreductase, is also required for hypoxic modulation of Kv4.2 [32].

Discovery of the molecular components constituting the oxygen-sensitive potassium channels and their oxygen sensors might help to develop understanding of important disorders, including pulmonary artery hypertension and persistent ductus arteriosus.

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