Localized alveolar hypoxia causes constriction of the small resistance pulmonary arteries, thus diverting the desaturated, mixed-venous blood to better ventilated areas of the lung. Although modulated by endothelial vasoactive substances, the constrictor response to hypoxia is intrinsic to the smooth muscle cell. Ion channels are important elements in two of the three components of the response. Hypoxia inhibits several potassium channels (voltage-gated and TASK), leading to membrane depolarization and calcium entry through L-type channels. It also causes release of calcium from the sarcoplasmic reticulum, with consequent repletion through store-operated calcium channels. Finally, the effect of the rise in cytosolic calcium is amplified by enhanced calcium sensitivity of the actin/myosin interaction, achieved by the hypoxia-induced increase in Rho-kinase activity. The change in oxygen tension that stimulates these three “executive” components is signaled by a change in the redox status of the smooth muscle cell and probably by downstream changes in G-proteins.

Ion channels also play a critical role in the vascular remodeling that results in chronic hypoxic pulmonary hypertension, seen when all the pulmonary vascular bed is hypoxic, at high altitude and in patients with chronic lung diseases. The same inhibition of potassium channels and influx of calcium results in high cytosolic levels of potassium and calcium. These, respectively, lead to inhibition of apoptosis and an increase in cellular proliferation. A better understanding of the pathophysiology of hypoxic pulmonary vasoconstriction and vascular remodeling will enable the design of better treatments for hypoxic and other forms of pulmonary hypertension.

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1. Introduction

Ion channel proteins probably exist in all life forms. Of these, the potassium channels form the largest and most diverse group, being present in both prokaryotes and eukaryotes [1]. An example of the conservation of potassium channel structure is provided by the remarkable homology in the amino acid sequence of voltage-sensitive potassium (Kv) channels in Drosophila and mammals [2]. Given the early appearance of ion channels in evolution and the generation of oxygen, starting about three billion years ago, it is not surprising that changes in oxygen levels might alter the gating of some ion channels. For instance, the reactive oxygen species, hydrogen peroxide, applied to three cloned Kv channels, expressed in Xenopus oocytes, increases the outward potassium current (I\text{K}) [3], indicating one potential link. Similarly, in a life form as simple as Escherichia coli, two K+ channels are known to be gated by the redox status of glutathione, the principal cellular redox couple [4].
Hypoxic pulmonary vasoconstriction (HPV) is a physiological response of small pulmonary arteries that diverts mixed-venous blood away from hypoxic alveoli, thus optimizing the matching of perfusion and ventilation and preventing arterial hypoxemia. When only a small region of the lung is hypoxic, HPV can occur without significant effect on pulmonary arterial pressure [5]. However, when hypoxia is generalized, as seen with many lung diseases and in high-altitude exposure, the subsequent pulmonary vasoconstriction contributes to pulmonary hypertension, heart failure and death. Although modulated by endothelium, hypoxia appears to activate mechanisms intrinsic to the pulmonary vascular smooth muscle cells, as HPV can be demonstrated in these isolated cells. Oxygen-sensitive ion channels, like K⁺ and Ca²⁺ channels, expressed in the pulmonary arterial smooth muscle cells are widely accepted to determine the vascular tone in response to hypoxia. We focus on the roles of these ion channels in acute pulmonary vasoconstriction and in chronic hypoxia-induced pulmonary hypertension.

1.1. The oxygen-sensing system

There are a variety of tissues that sense the oxygen level in different strategic locations in the body. These include the type I cells of the carotid body, neuroepithelial bodies in the lungs, (NEBs), chromaffin cells of the fetal adrenal medulla, and smooth muscle cells of the resistance pulmonary arteries, fetoplacental arteries, systemic arteries, and the ductus arteriosus. Together, they constitute a specialized homeostatic oxygen-sensing system. There is no doubt that changes in oxygen level alter the activity of some ion channels. This can be considered the executive limb of the response. The key to the executive mechanism, that is common to all these tissues, is the observation that a change in oxygen alters IK. This in turn changes membrane potential and determines the entry of calcium through the L-type calcium channel. The finding that hypoxia reduces IK was first reported in the type I cell of the carotid body [6]. Subsequently, similar observations were made in NEBs [7], adrenal chromaffin cells [8], smooth muscle cells of the resistance pulmonary arteries [9] and fetoplacental arteries [10]. At birth, as the oxygen tension rises, the pulmonary arteries dilate and the ductus arteriosus constricts. In the smooth muscle cells of the ductus it is normoxia, not hypoxia, that inhibits IK and depolarizes the cell membrane [11]. This opposite control of the K⁺ channel emphasizes the importance of this mechanism in the response of these tissues to changes in oxygen. In the case of the vessels, two other mechanisms participate in the response that have not been described in the type I cell of the carotid body or in NEBs. These are the release of calcium from the sarcoplasmic reticulum of the smooth muscle cell [12], associated with repletion through the store-operated channels (SOC) [13], and increased calcium sensitivity associated with inhibition of myosin light chain phosphatase by Rho-kinase [14,15].

2. Acute hypoxic pulmonary vasoconstriction

In fetal life, pulmonary vascular resistance is high and oxygenated blood returning from the placenta flows through the foramen ovale and ductus arteriosus, largely bypassing the lungs. If the oxygen level in the fetus is raised by increasing maternal oxygenation, the fetal pulmonary vascular resistance falls, demonstrating that normally in the fetus active hypoxia-induced vasoconstriction contributes to the high resistance [16]. At birth, lung expansion and rising oxygen levels cause a rapid drop in pulmonary vascular resistance and subsequent remodeling of the resistance pulmonary arteries.

In the neonate and adult, alveolar hypoxia, in the physiologic range (from 40 to 80 mm Hg), causes vasoconstriction of the resistance pulmonary arteries (PA) [17]. This hypoxic pulmonary vasoconstriction (HPV) reduces perfusion of poorly ventilated areas of the lung and consequently decreases the shunting of desaturated mixed-venous blood to the left side of the heart and systemic circulation. While mediators such as endothelin and serotonin undoubtedly modulate HPV, the ability to contract in response to hypoxia resides in the smooth muscle cells (SMC) of the resistance pulmonary arteries (PA) [18], containing both sensors and effectors of HPV. On the basis of current research, it can be postulated that a redox-based O₂-sensor signals HPV, involving hypoxia-induced changes in redox couples (a shift toward reduced versus oxidized glutathione, GSH/GSSG, or NADH/NAD) and/or mitochondrial-derived- and/or nicotinamide adenine dinucleotide phosphate oxidase (NAD(P)H) oxidase-derived ROS generation as outlined later on. The proposed functional effectors include 1) inhibition of O₂-sensitive K⁺ channels leading to membrane depolarization, 2) opening of the L-type Ca²⁺ channels (either as a result of K⁺ channel inhibition or as a primary event), 3) release of Ca²⁺ from ryanodine-sensitive stores in the PASMC sarcoplasmic reticulum (SR) and repletion through the SOC, 4) Ca²⁺-sensitization involving Rho-kinase.

2.1. Decreased K⁺ channel activity in acute HPV

The membrane potential of pulmonary artery smooth muscle cells (PASMC) is an important regulator of arterial tone. These cells have a resting membrane potential of around −65 to −50 mV in vitro, close to the predicted equilibrium potential for potassium (K⁺) ions. At least four classes of K⁺ channels have been identified in PASMCs: voltage-dependent potassium channels (Kᵥ) [19–21], calcium-activated potassium channels (KᵥCa) [22,23], ATP-sensitive potassium channels (Kₐ₅P) [24] and two-pore domain K⁺ channels [25,26]. The decrease in K⁺ conductance in PASMCs leads to cell depolarization that enhances the open probability of L-type Ca²⁺ channels in smooth muscle cells, causing Ca²⁺ entry, and vasoconstriction (Fig. 1).
In the fetus and neonate it seems that the membrane potential of the PA smooth muscle cells (SMCs) is largely controlled by calcium-sensitive potassium (KCa) channels that are inhibited by hypoxia [16]. With maturation, the control of membrane potential shifts to Kv and TASK-1 K+ channels [27,25]. This is similar to the shift in the type I cells of the carotid body from oxygen-sensitive KCa in the neonate, to Kv and TASK-like channels in the adult [28–31]. In the adult animal the Kv channels are found predominantly in the small resistance PA, while the KCa channels are more common in the large, conduit PA [32]. It seems likely that both TASK-1 and Kv channels contribute to the hypoxic depolarization of the adult PASMC [33,26].

Nine families of Kv channels α-subunits are recognized from cloning studies (Kv1–9) [34,35], each with subtypes (e.g. Kv1.1–1.6). These channels often display differences in voltage sensitivity, current kinetics and steady-state activation and inactivation [36]. Kv channels exist as tetramers formed by α-subunits combining to form functional channels. Not only can identical α-subunits combine to form a functional channel, but distinct α-subunits can also combine to form functional heteromeric channels with unique properties [38]. Accessory β-subunits can combine with Kv α-subunits to add even more diversity to Kv channel function [39]. Kv β-subunits may play a role as a cellular redox sensor because they appear to confer O2-sensitivity on the Kv4.2 channel in heterologous expression systems [40]. This information suggests the potential role of Kv β-subunits not only in the functional modulation of Kv α-subunits but also in the PASMC response to hypoxia.

Based on patch-clamp recordings with specific Kv antibodies, studies in expression systems, or the use of reverse transcription polymerase chain reaction, the potential candidate Kv channel α-subunits that could form O2-sensitive channels are Kv1.2 [41,42], Kv1.5 [41,43], Kv2.1 [44,43,42], Kv3.1 [45] and Kv9.3 [44,42]. All these O2-sensitive channels could be detected in PASMCs at the mRNA level [46]. At present most of the evidence points towards a role for Kv1.5 and Kv2.1. When antibodies to these two channels are dialysed from the patch pipette into a PASMC, the membrane potential is depolarized and the residual IK has little oxygen sensitivity [43]. Mice that lack Kv1.5 have less oxygen-sensitive IK and demonstrate diminished HPV [47] (Fig. 2). Unlike other Kv channel proteins, Kv1.5 shows more protein in the small resistance PA than in conduit PA [48]. One additional experiment reinforces the role of Kv1.5 in the executive mechanism of HPV. It is well established that isolated perfused lungs taken from animals previously

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**Fig. 1.** Proposed schematic presentation of ion channels involved in acute HPV in pulmonary artery smooth muscle cells (PASMC). In PASMC hypoxia, in part due to altered release of reactive oxygen species (ROS) and/or changes in redox status inhibits voltage-gated K+ channels (Kv) and two-pore domain K+ channels (K2P), leading to the depolarization of the membrane (Em) and activation of voltage-operated L-type Ca2+ channels (VOCC). Depletion of Ca2+ from the sarcoplasmic reticulum opening of SOC allows for Ca2+ influx, termed capacitative calcium entry. Intracellular [Ca2+]i is also increased by hypoxia through the activation of store-operated (SOC) or receptor-operated (ROC) Ca2+ channels. The hypoxia-induced rise in [Ca2+]i then triggers PASMC contraction. ETC electron transport chain, NAD(P)H oxidase; SR sarcoplasmic reticulum; Ryr-R ryanodine receptors, IP3 inositol-1,4,5-triphosphate receptor; KCa Ca2+-sensitive K+ channels.
exposed to chronic hypoxia, have markedly diminished HPV [49]. The remarkable observation is that acute HPV can be restored by aerosol transfection of Kv1.5 [50].

While the studies described above indicate the involvement of Kv channels, HPV persists in isolated lungs treated with 5 mM 4-aminopyridine and 10 mM TEA, as inhibitors of Kv and KCa channels (Fig. 3; similar results have been published by Hasunuma et al. [51]), thus suggesting the involvement of other mechanisms (i.e. other K+ channels, store- or receptor-operated Ca2+ increase or Ca2+-sensitization by hypoxia). In the carotid body, hypoxia may initiate membrane depolarization of the type I cells by inhibition of the voltage-independent TASK-like potassium channels that conduct a basal leak current at resting membrane potential [31]. Although this group of K+ channels, so-called background K+ channels, escaped detection at the molecular level for many years, it has now become clear that the newly identified KCNK family of K+ channel subunits also controls resting membrane potential in PASMCs [52,25]. In addition, substantial evidence has recently emerged that point to the contribution of the instantaneous non-inactivating TASK-1 channels in O2-sensing in human PASMCs as shown in Fig. 3 [26].

2.2. Increased Ca2+ channel activity in acute HPV

An increase of the cytosolic Ca2+ concentration ([Ca2+]cyt) is necessary to elicit hypoxic constriction of the resistance
PA. In PASMCs, $[\text{Ca}^{2+}]_{\text{cyt}}$ is increased by $\text{Ca}^{2+}$ influx through $\text{Ca}^{2+}$-permeable channels and/or by $\text{Ca}^{2+}$ mobilization from intracellular $\text{Ca}^{2+}$ stores (e.g., endoplasmic/ sarcoplasmic reticulum). A major mechanism for increasing the concentration of $\text{Ca}^{2+}$ in the cytoplasm during hypoxia is the influx of extracellular $\text{Ca}^{2+}$ across the cell membrane in PASMCs [53]. The best characterised pathway of $\text{Ca}^{2+}$ entry into PASMCs is through nifedipine-sensitive L-type voltage-operated $\text{Ca}^{2+}$ channels (VOCC), regulated by the resting membrane potential. However, the incomplete inhibition of HPV by nifedipine indicates the existence of additional $\text{Ca}^{2+}$ entry pathways in PASMCs, distinct from VOCCs, namely receptor-operated $\text{Ca}^{2+}$ channels (ROCs), which are activated by agonists and store-operated $\text{Ca}^{2+}$ channels (SOCs), which are opened by depletion of $\text{Ca}^{2+}$ from the SR.

### 2.2.1. Voltage-operated L-type $\text{Ca}^{2+}$ channels (VOCC)

The involvement of L-type calcium channels in HPV was suspected before it was confirmed by whole-cell patch clamp studies. The L-type calcium channel agonist BAY K8644 had little effect on normoxic pulmonary vascular tone in the isolated perfused lung but dramatically increased HPV [54,55]. L-type calcium channels are present in both conduit and resistance pulmonary arteries but the density of these channels is about twice as great in the smooth muscle cells of the resistance arteries [56]. Although the L-type channels are homogeneous, in the sense that they are all inhibited by nifedipine, influx of calcium into the majority of the smooth muscle cells of the resistance arteries is enhanced by hypoxia, while in the smooth muscle cells of the conduit cells it is inhibited [56]. Thus the L-type channels in the conduit arteries behave, in response to hypoxia, like those of systemic arteries [57].

### 2.2.2. Store-operated channels (SOC)

Although most of the increase in cytosolic calcium responsible for HPV comes from outside the smooth muscle cell, induced by membrane depolarization via L-type $\text{Ca}^{2+}$ channels, $\text{Ca}^{2+}$ released from the sarcoplasmic reticulum through ryanodine and IP$_3$-sensitive receptors also contributes to HPV. If the calcium in the sarcoplasmic reticulum is depleted by agents such as ryanodine, then subsequent HPV is diminished [12,58,59]. How is the calcium in the sarcoplasmic reticulum restored? The observation that hypoxia still causes contraction of small pulmonary arteries in the presence of 80 mM KCL and nifedipine indicates that there are pathways for calcium entry in addition to the L-type calcium channel [13]. Release of calcium from the sarcoplasmic reticulum activates capacitative calcium entry through store-operated channels, likely to be coded by transient receptor potential (TRP) genes [60,61]. In pulmonary artery smooth muscle cells, after depletion of calcium by cyclopiazonic acid, in the presence of L-type calcium channel blockade with nifedipine, the influx of calcium is greatly enhanced under hypoxic compared to normoxic conditions [61]. This influx can be inhibited by blockers of the store-operated channels, such as SKF-96365, nickel and La$^{3+}$. The same blockers can both prevent and reverse HPV in the isolated perfused lung [62]. These studies provide convincing evidence of the importance of store-operated channels in the mechanisms responsible for HPV.

### 2.3. Rho-kinase and $\text{Ca}^{2+}$ sensitization

Although this review is focused on ion channels, calcium sensitization plays an important part in HPV and enhances the effect of the increase in cytosolic calcium that we have noted to occur by calcium entry through L-type and store-operated channels, and by calcium release. The interaction of actin and myosin that causes pulmonary artery smooth muscle contraction, in response to any stimulus including hypoxia, is initiated by phosphorylation of myosin light chain, induced by myosin light-chain kinase. The interaction is terminated by dephosphorylation, induced by myosin light-chain phosphatase. Hypoxia acts through the small G-protein, RhoA to stimulate Rho-kinase, that in turn inhibits myosin light-chain phosphatase and increases HPV [63]. What is the signaling sequence that permits hypoxia to inhibit potassium channels, release calcium from the sarcoplasmic reticulum, replete calcium through the store-operated channels and increase RhoA?

### 2.4. Oxygen signaling to ion channels

At birth, the resistance pulmonary arteries dilate and the ductus arteriosus contracts in response to increasing oxygen levels. In both vessels, substances such as endothelin and prostaglandin $\text{F}_2\alpha$ cause contraction. Only redox-active agents and mitochondrial inhibitors, (such as rotenone), have opposite effects on tone in the pulmonary arteries and ductus [64–66]. The reducing agent, dithiothreitol (DTT), inhibits K$^+$ current, causes membrane depolarization, increases cytosolic calcium and induces vasoconstriction in the resistance PA, while doing exactly the opposite in the ductus arteriosus, thus mimicking hypoxia. On the other hand, the oxidizing agent DTNB mimics the effect of oxygen in each of these respects and causes relaxation in the PA and constriction of the ductus [66]. Rotenone and hypoxia decrease the production of reactive oxygen species (ROS) in both PA and ductus smooth muscle cells but only decrease K$^+$ current and cause membrane depolarization in the PA while doing the opposite in the ductus [65]. Concordant with these data, exogenous hydrogen peroxide, inhibits K$^+$ current and causes membrane depolarization in the ductus smooth muscle cells, while removal of endogenous hydrogen peroxide does the opposite [67,65]. These observations favor the hypothesis that redox changes may transfer the signal from a change in oxygen tension to an alteration in ion channel gating. It remains to be determined whether the redox change involves redox couples such as NAD(P)/NAD (P)$_2$H or GSSG/GSH, downstream metabolites like cyclic ADP-ribose [68] and/or alterations in the levels of ROS [69–
71]. In the case of ROS, there is still no consensus on whether ROS go down with hypoxia [72], go up with hypoxia [73], or might do both simultaneously in different compartments [74].

As noted earlier, after chronic hypoxia, HPV is diminished but it can be restored by aerosol transfection of K<sub>1.5</sub> [50]. Similarly, after chronic normoxia, normoxic contraction of the ductus is diminished and it can be partially restored by transfection with the same K<sub>1.5</sub> [75]. The significance of these observations, when considered together, is that the opposite behavior of the K<sup>+</sup> current is not a property of the α-subunit of the K<sub>v</sub> channel. The opposite behavior could relate to a different β-subunit in each vessel or to different G-proteins that are more proximal in the signaling pathway between the redox change and the channel. An increase in the small G-protein, RhoA, mediates the effects of hypoxia, signaled through a redox change, to alter PA endothelial function [76]. An increase in RhoA also mediates the changes in calcium sensitivity in response to hypoxia, as discussed earlier. An increase in RhoA is an essential component of the signaling pathway by which a muscarinic receptor agonists cause tyrosine phosphorylation of the amino terminus of K<sub>1.2</sub> channel protein, leading to suppression of the potassium current [77]. As noted earlier, K<sub>1.2</sub> is oxygen-sensitive, as is K<sub>1.5</sub>, which is also inhibited by tyrosine phosphorylation at a specific point in the channel protein [78]. Thus, RhoA can be identified as a modulator of K<sub>v</sub> channel activity and as a link in the process of G-protein-coupled receptor-mediated tyrosine kinase suppression of K<sup>+</sup> channels. The use of the Rho-kinase inhibitor, Y27632, suggests that Rho-kinase, which increases downstream from an increase in RhoA, may also modulate K<sup>+</sup> and Ca<sup>2+</sup> currents. In rat cerebral arteries, Y27632 abolished the ability of the pyrimidine nucleotide UTP to inhibit K<sub>v</sub> current. The effect of UTP on K<sub>v</sub> current was also prevented by inhibition of RhoA by C3 exoenzyme. These studies imply, if the inhibitors are specific, that RhoA/Rho-kinase mediates the K<sub>v</sub> channel inhibition [79]. In addition, the use of Y27632 and another Rho-kinase inhibitor, fasudil, in smooth muscle cells from systemic arteries, suggests that Rho-kinase is involved in the activation of receptor-mediated calcium entry, distinct from voltage- or store-operated channels [80]. In view of these studies, it would not be surprising if the G-proteins and Rho-kinase were not only involved in hypoxic calcium-sensitization but if they also conveyed the hypoxic signal to the K<sup>+</sup> channels and possibly to the SR and SOC, thus providing a key link in the chain between changes in oxygen tension and ion channels.

### 3. High-altitude pulmonary edema (HAPE)

Acute exposure to environmental hypoxia caused by the rapid movement of native sea level dwellers to high altitude, may lead to the development of increased pulmonary vascular resistance and pulmonary hypertension [81,82]. During the early period of hypoxic exposure, the increase in pulmonary arterial pressure is largely due to HPV, as mentioned earlier. It has been suggested that the HPV may be patchy, with the result that some pulmonary capillaries are exposed to higher pressure and flow [83]. This might cause stress failure of the capillary wall, a leak of high-protein edema fluid and erythrocytes, resulting in high-altitude pulmonary edema (HAPE). HAPE is a potentially fatal condition that typically occurs 2 to 4 days after ascent to altitudes above 3000 m [84]. The considerable individual variability in the magnitude of HPV between humans, based on genetics and adaptive mechanisms, could explain the observation that the incidence of HAPE is approximately 2–15%, although the incidence of subclinical fluid accumulation may be higher [85]. HAPE can pose a problem for mountaineers, skiers and troops deployed at high altitude.

The main question to be addressed here is whether specific ion channels or ion transporters are involved in the mechanism. Can altered ion channel expression or activity account for the susceptibility of some individuals to HAPE? Extensive evidence documents that HPV can be attributed to the inhibition of K<sup>+</sup> channels, leading to membrane depolarization increases, in Ca<sup>2+</sup> influx via voltage-dependent Ca<sup>2+</sup> channels and enhanced capacitative Ca<sup>2+</sup> entry in PASMCs as described in detail above. On the other hand, hypoxia-dependent K<sub>a</sub> channels have been found in alveolar epithelial cells [86]. The closing of a K<sup>+</sup> conductance in alveolar epithelium by hypoxia decreases alveolar fluid clearance [87] leading to fluid retention and contributing to lung edema. A further study on alveolar epithelial cells confirms that hypoxia down-regulates the expression and activity of epithelial Na<sup>+</sup> channels (ENaC) and Na,K-ATPase, thus reducing salt and water clearance [88]. This suggests that susceptibility to HAPE could also involve decreased expression and activity of epithelial Na<sup>+</sup> channels and Na,K-ATPase, with a subsequent decrease in Na<sup>+</sup> reabsorption by alveolar epithelium. In addition to epithelial effects, hypoxia also causes an increase in endothelial permeability, through an increase in RhoA, formation of stress fibers and dispersion of adherens junctions [76].

Studies designed to define the role of ion channels and transporters in HAPE-susceptible humans are limited, due to the lack of direct access to cells that may be involved in HAPE. However, individuals who develop HAPE do show an abnormal increase in pulmonary artery pressure during brief or prolonged hypoxic exposure [89] and a patchy peripheral distribution of edema on chest X-ray and computerized tomography scans. Thus, one hypothesis is that regional heterogeneity in HPV is due to an inhomogeneous expression/function of O<sub>2</sub>-sensitive ion channels [90,48] and could this be etiologic feature of HAPE. A related possibility is based on the observation that hypoxia causes constriction of small pulmonary veins as well as arteries [17]. An increase in downstream resistance to blood flow leaving the capillary bed would predispose to edema. An increase in pulmonary wedge pressure, a measure of
pressure in the capillary bed, has been noted in patients with HAPE [91]. As K⁺ channels modulate pulmonary venous tone [92] it is not implausible that they may be oxygen-sensitive and render some individuals more susceptible to HAPE.

4. Chronic hypoxic vasoconstriction–hypoxic pulmonary hypertension

Chronic hypoxia related to severe emphysema, chronic obstructive pulmonary disease (COPD), obstructive sleep apnoea (normobaric hypoxia), as well as living at high altitude (hypobariic hypoxia), may result in a sustained increase in pulmonary vascular resistance. Regardless of the pathologic mechanism, elevated pulmonary vascular resistance and sustained pulmonary hypertension put an excessive burden on the right ventricle and eventually lead to the clinical syndrome of right heart failure with systemic congestion and inability to increase right ventricular output in response to peripheral demand on exercise. Consequently, a progressive loss of exercise capacity, quality of life, and life expectancy are potential consequences for individuals affected by these conditions.

Chronic high-altitude disease associated with excessive pulmonary hypertension and right heart failure was first described in Colorado cattle (brisket disease) [93]. It was recognized that susceptibility to brisket disease and hyper-reactive HPV was inheritable [94]. This altitude-induced hypertension offers no obvious benefit and may be maladaptive. In fact, the Tibetan population and high-altitude-adapted animals (e.g. Tibetan sheep), populations that are genetically best adapted to high altitude, have weak or absent HPV [95,96], demonstrating that evolution has selected against the reactive pulmonary vascular bed at altitude. Histopathology shows that experimental chronic hypoxia at high altitude predominantly induces medial hypertrophy in pulmonary arteries, which can be almost completely reversed after a few weeks back at sea level [97]. The actual incidence of pulmonary hypertension in severe emphysema, COPD and obstructive sleep apnoea is not known, because patients are not screened systematically. However, evidence for right ventricular hypertrophy found at autopsy of patients with COPD or direct measurement of pulmonary arterial pressure in different studies estimates the incidence at up to 60% [98,99] and the mortality rate correlates with the severity of pulmonary hypertension. In contrast to the hypobaric hypoxia-induced pulmonary hypertension, pathologic studies demonstrate a major remodeling of all layers of the resistance pulmonary arteries, with intimal changes being the most prominent.

4.1. Ion channels are involved in decreased apoptosis and increased cell proliferation under chronic hypoxia

The precise control of the balance between proliferation and apoptosis is important for adaptation to changed oxygenation, flow and pressure conditions within the resistance vessels of the lung. In chronic hypoxia this balance seems to be disturbed. The maladaptive response, increased PASMC proliferation and decreased apoptosis, results in vessel wall thickening, vascular remodeling and consequently in sustained pulmonary hypertension. If the enhanced proliferation and inhibited apoptosis lead to vascular remodeling, how might they occur?

4.2. Down-regulation of K⁺ channels in chronic hypoxic pulmonary hypertension

Because K⁺ channels are important determinants of vascular tone control and the proliferative status of vascular smooth muscle cells, the roles of K⁺ channels and membrane potential have been investigated in several animal models of chronic pulmonary hypertension. Shortly after the first reports of the effect of acute hypoxia on K⁺ channels, Smirnov et al. demonstrated that PASMCs of rats raised for four weeks in an hypoxic environment have reduced Kᵅ current compared with normoxic rats [100]. The resting potential of PASMCs from chronically hypoxic animals was significantly more positive [100]. It was proposed that the observed reduction in Kᵅ current amplitude was as a result of decreased channel expression [101]. The first evidence for down-regulation of specific Kᵅ-channel α-subunits (Kᵅ1.2 and Kᵅ1.5) in cultured rat PASMCs under chronic hypoxia was provided by Wang et al. [41]. Decreased mRNA expression of Kᵅ1.1, Kᵅ1.5, Kᵅ2.1, Kᵅ4.3 and Kᵅ9.3 α-subunits in cultured rat PASMCs under hypoxia was confirmed by other groups [102,103]. In contrast, chronic hypoxia fails to inhibit the expression of Kᵅ channels α- or β-subunits examined in mesenteric arterial SMCs [102,103]. This suggests that the response is specific to PASMCs and therefore selective for the pulmonary circulation. Two animal models emphasize the importance of Kᵅ channels in the pulmonary vascular response, the chronically hypoxic animal model and the Kᵅ1.5 knockout mouse model. Studies investigating changes in protein expression and function of Kᵅ α-subunits show down-regulation of Kᵅ1.2 and Kᵅ1.5 and a decrease in the oxygen-sensitive component of Iᵅ in freshly isolated PASMCs from chronically hypoxic animals [104]. A more recent study demonstrated that a period of hypoxia less than 24 h causes reduction of Kᵅ1.2, Kᵅ1.5 and Kᵅ2.1 mRNA with consequent membrane depolarization and increased [Ca²⁺]ᵅ in chronically hypoxic rats [105]. An elegant experiment by Pozeg and co-workers indicates that enhancing expression of Kᵅ1.5, via Kᵅ1.5 adenoviral gene transfer, restores Kᵅ expression, O₂-sensitive K⁺ current, and HPV [50]. Finally, Kᵅ1.5 knockout mice show impaired HPV and reduced O₂-sensitive K⁺ current in PASMC [47] (Fig. 2). These data provide strong evidence for the role of Kᵅ channels in the chronic pulmonary vascular response to hypoxia. The reduction of the Kᵅ channel activity and the consequent membrane depolarization appears to be involved in the development of chronic hypoxic pulmonary hypertension by
mediating pulmonary vasoconstriction and vascular remodeling through increased $[Ca^{2+}]_{cyt}$ in PASMCs (Fig. 4).

Many hypotheses have been put forth to explain the chronic hypoxia-induced inhibition of $K_v$ channels in PASMCs. These include the production of reactive oxygen species, redox status, oxygen-sensitive regulatory molecules that are on or closely associated with the $K^+$ channels, the activity of hemoproteins such as cytochrome P-450 or NADPH oxidase; and/or direct transcriptional regulation, similar to proposed regulatory mechanisms in acute HPV. One mechanism that might explain $K_v$ channel modulation during chronic hypoxia is the direct influence of the cytosolic redox shifts on $K_v$ channels, affecting redox-sensitive thiol groups in regulatory subunits, causing the channels to close. Chronic hypoxia has been reported to increase the reduced glutathione levels before $K_v1.5$ and $K_v2.1$ channel protein is decreased [104]. There is accumulating evidence that chronic hypoxic exposure is associated with protein phosphorylation and/or redox modulation of transcription factors, including hypoxia-inducible factor-1 (HIF-1), that is composed of HIF-1$\alpha$ and HIF-1$\beta$ subunits [106,107]. HIF-1$\beta$ is expressed constitutively in many cells. HIF-1$\alpha$ is rapidly degraded during normoxia due to its hydroxylation by prolyl hydroxylase, but stabilized during hypoxia via inhibition of that process, allowing it to heterodimerize and activate transcription [108]. HIF-1$\alpha$ is expressed in the lung, regulated by the inspired $O_2$ concentration and is reported to play a role in hypoxia-induced pulmonary vascular remodeling [108,109]. In comparison with wild-type mice, the muscularization of pulmonary arterioles is significantly decreased in heterozygous HIF-1$\alpha^{+/-}$ mice exposed to hypoxia for three weeks. Furthermore, other studies showed that partial deficiency of HIF-1$\alpha$ is sufficient to reduce hypoxia-induced depolarization, $K_v$ current density, and PASMC hypertrophy [110].

It has recently been reported that overexpression of c-jun gene significantly decreases whole cell $K_v$ current, down-regulates the mRNA expression of the $K_v1.5$ channel $\alpha$-subunit and accelerates $K_v$ current inactivation in cultured PASMCs [111]. Significant membrane depolarization and stimulated SMC proliferation in these cells have also been demonstrated, caused by overexpression of the gene [111]. There is a large body of data indicating that the c-jun family of genes is inducible by hypoxia [112–114]. Yu et al. interpreted their results to suggest that the c-jun may indirectly down-regulate transcription of $K_v1.5$ gene and decrease $K_v$ current [111]. The resultant membrane depolarization then could increase $[Ca^{2+}]_{cyt}$ and induce cell proliferation.
Knowledge of the mechanism of decreased apoptosis in vascular remodeling and the signaling pathways that mediate this cellular response to sustained hypoxia has developed rapidly in the last decade. In PASMC any changes in K⁺ efflux or influx via ion channels and transport mechanisms will influence the regulation of cellular volume and apoptosis. Several studies have proposed that apoptosis is mediated, at least in part, by loss of K⁺ channels [115–117]. In PASMC, the selective loss of sarcolemmal K⁺ channels like Kᵥ during chronic hypoxia decreases transmembrane K⁺ efflux, which would then increase cytosolic K⁺ and thus reduce apoptosis. In contrast, increased transmembrane K⁺ influx (K⁺ channels open and/or the number of functional K⁺ channels increased due to upregulation of K⁺ channel gene expression) enhances cell volume decrease and causes apoptosis. Accordingly, upregulation of K⁺ channels could be a key target in the treatment of pulmonary vascular diseases where the balance between cell proliferation and apoptosis has been disturbed e.g. chronic pulmonary arterial hypertension [50].

4.3. Elevated cytoplasmic Ca²⁺ in chronic hypoxia-induced PAH

An increase in intracellular Ca²⁺ plays an obligatory role in the pulmonary pressor response to hypoxia. Although the precise mechanisms involved in sustained hypoxia-induced Ca²⁺ influx across the plasma membrane are still unclear, it is now apparent that voltage-independent Ca²⁺ channels are most affected in vascular smooth muscle cells. In the pulmonary vasculature, Ca²⁺ influx through voltage-independent Ca²⁺ channels involves two possible pathways: 1) store-operated Ca²⁺ channels (SOC) activated by depletion of Ca²⁺ from intracellular stores and 2) receptor-operated Ca²⁺ channels (ROC) activated by interaction of agonist with membrane receptors. The characterisation of the transient receptor potential (TRP) family of cation channel proteins has given fresh impetus to the molecular identification of membrane potential-independent Ca²⁺ influx in cells involved in the pulmonary hypoxic response. In PASMCs, the first evidence for role of the Ca²⁺ influx due to SOCs and ROCs in chronic pulmonary vasoconstriction, was provided by Hong et al., Lin et al. and Wang et al. [118–120]. The elegant study of Lin et al. demonstrated that both store-(TRPC1) and receptor-(TRPC6) operated channels of PASMCs are upregulated by chronic hypoxia and contribute to the enhanced vascular tone in hypoxic pulmonary hypertension [120]. It is now apparent that elevated [Ca²⁺]cyt stimulates PASMC proliferation, associated with a significant increase in mRNA and protein expression of TRPC1 and TRPC6 [121–123]. The resulting elevation of [Ca²⁺]cyt represents another mechanism for hypoxia-mediated pulmonary vasoconstriction and PASMC hypertrophy and proliferation. Additionally, Fantozzi et al. recently reported that in human PAEC chronic hypoxia significantly upregulates TRPC4, augments the activity of SOC, enhances the amplitude of capacitative Ca²⁺ entry and leads to elevated [Ca²⁺]cyt [124]. This study also demonstrated that the increased [Ca²⁺]cyt enhances the transcription factor-activating protein (AP)-1 DNA binding activity in PAEC under sustained hypoxia, which modulates expression of genes involved in cell proliferation. Therefore, upregulation of TRPC channels appears to be a critical mechanism by which growth factors mediate PASMC proliferation and it may have an important impact on the development of chronic hypoxia-induced PAH.

5. Conclusion

The three executive components of HPV are generally agreed. It also seems likely that the rise in cytosolic potassium and calcium in the PASMC causes decreased apoptosis and increased cellular proliferation respectively, and contributes to the remodeling seen in chronic hypoxic pulmonary hypertension. However, the signalling involved in both acute and chronic ion channel responses to hypoxia is still debated. In the acute HPV it is still considered that changes in ROS and redox status are important and we believe that G-proteins, such as RhoA/Rho-kinase may telegraph the redox changes to the SR and ion channels. In chronic hypoxic pulmonary hypertension, HIF-1α and cytosolic calcium and potassium may play significant roles, although this is not universally accepted.

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