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High Altitude Pulmonary Hypertension: Role of K⁺ and Ca²⁺ Channels

Carmelle V. Remillard, PhD and Jason X.-J. Yuan, MD, PhD*

From the Department of Medicine, School of Medicine, University of California, San Diego, La Jolla, CA 92093-0725

Abstract

Global alveolar hypoxia, as experienced at high-altitude living, has a serious impact on vascular physiology, particular on the pulmonary vasculature. The effects of sustained hypoxia on pulmonary arteries include sustained vasoconstriction and enhanced medial hypertrophy. As the major component of the vascular media, pulmonary artery smooth muscle cells (PASMC) are the main effectors of the physiological response(s) induced during or following hypoxic exposure. Endothelial cells, on the other hand, can sense humoral and haemodynamic changes incurred by hypoxia, triggering their production of vasoactive and mitogenic factors that then alter PASMC function and growth. Transmembrane ion flux through channels in the plasma membrane not only modulates excitation-contraction coupling in PASMC, but also regulates cell volume, apoptosis, and proliferation. In this review, we examine the roles of K⁺ and Ca²⁺ channels in the pulmonary vasoconstriction and vascular remodeling observed during chronic hypoxia-induced pulmonary hypertension.

Keywords

vascular smooth muscle; ion channels; proliferation; membrane potential; chronic hypoxia

Introduction

The global alveolar hypoxia associated with high altitude causes elevated pulmonary vascular resistance (PVR) and increased pulmonary arterial pressure (PAP), which may eventually cause right heart failure. Part of the increase in PAP is due to a phenomenon termed “hypoxic pulmonary vasoconstriction” (HPV) whereby oxygen sensors located in the pulmonary vasculature detect the drop in O₂ tension (PO₂) in the air and ultimately lead to vasoconstriction of small pulmonary arteries and arterioles (Fig. 1). The goal of HPV is to match ventilation with perfusion and to reduce arterial hypoxemia by diverting blood flow away from poorly ventilated (or oxygenated) regions. Persistent alveolar hypoxia causes pulmonary arterial hypertension (PAH) as a result, at least in part, of sustained HPV. However, chronically hypoxic rats (McMurtry et al., 1978), and high-altitude adapted animals (yak, Tibetan sheep) and humans (native Tibetans) have weak or absent HPV (Durmowicz et al., 1993; Moore et al., 1998a). Indeed acute HPV is selectively downregulated after as little as 3 h of hypoxic exposure (Greenlees et al., 1984) in simulated altitude animal models. Although the reason for this loss of HPV is unclear, the data suggest that there is increased activity of some endogenous vasodilator or abnormality in the mechanism(s) that couple hypoxia to pulmonary artery

*To whom correspondence should be addressed Jason X.-J. Yuan, M.D., Ph.D. Div. of Pulmonary and Critical Care Medicine University of California, San Diego 9500 Gilman Drive, MC 0725 La Jolla, CA 92093-0725 Tel: (858) 822-6532 Fax: (858) 822-6533 E-mail: cremillard@ucsd.edu

Carmelle V. Remillard, Ph.D. Div. of Pulmonary and Critical Care Medicine University of California, San Diego 9500 Gilman Drive, MC 0725 La Jolla, CA 92093-0725 Tel: (858) 822-6534 Fax: (858) 822-6531 E-mail: xiyuan@ucsd.edu

smooth muscle cells (PASMC) contraction. Recent studies have proposed that nitric oxide production might be responsible. Koizumi et al. found that endogenous production of nitric oxide and endothelial nitric oxide synthase (eNOS) activity were significantly enhanced in Tibetan sheep living adapted to high altitudes (Koizumi et al., 2004; Ruan et al., 2004). However, inhibition of voltage-dependent K^+ channels had no effect on pulmonary vascular tone and HPV in Tibetan sheep (Ishizaki et al., 2004) or in chronically hypoxic rats (Reeve et al., 2001). However, Archer's group found that chronic reduction of the cytosol due to decreased basal activated oxygen species levels and enhanced reduced glutathione levels occurred prior to K_v inhibition and K_v gene downregulation (which were only evident after 3 weeks of hypoxia) (Reeve et al., 2001). Therefore, they speculated that HPV may be attenuated in chronic hypoxia because of an impaired redox sensor rather than to impaired hypoxic response mechanisms.

Long before the latter findings were published (or even conceived of!), there were indications that the medial thickness of the small pulmonary arteries was highly correlated with the development of pulmonary hypertension. We now know that PAH induced by chronic alveolar hypoxia is also due to remodeling of the pulmonary vascular wall, primarily medial hypertrophy (Fig. 1). The latter occurs as a consequence of either increased PASMC hypertrophy and hyperplasia, or decreased PASMC apoptosis in the *tunica media*. Deregulated PASMC proliferation is a central theme for the vascular remodeling associated with chronic hypoxia-mediated PAH (Durmowicz et al., 1999). While some of the physiological manifestations are different, the mechanisms underlying the increased PAP associated with both acute and chronic hypoxia are nonetheless very similar. Some of the proposed mechanisms include ion channel dysfunction, changes in redox status, production of reactive oxygen (ROS) and nitrogen (RNS) species, inhibition of metabolism or mitochondrial oxidative phosphorylation, and O_2 sensing. Readers are invited to consult recent monologues which outline and describe these mechanisms more thoroughly (Raj et al., 2002; Mauban et al., 2005; Moudgil et al., 2005; Waypa et al., 2005).

Since hypoxia-induced PAH is reversed only partially with O_2 administration or hyperbaric exposure, it is apparent that pulmonary vascular remodeling is a long-term acquired physiological or pathophysiological response to decreased atmospheric PO_2 . There may even be a genetic component since high altitude-bred cattle with Brisket's disease have an inherited susceptibility to developing PAH and right heart failure (Grover et al., 1963; Will et al., 1975). However, the generalization that chronic hypoxia causes pulmonary arterial medial hypertrophy is not altogether correct. Such is the case with Himalayan highlanders who have successfully adapted to high-altitude living. Their thin-walled small pulmonary arteries show no evidence of medial hypertrophy or increased arteriolar muscularization, suggesting that they may be adapted to the hypobaric hypoxia (Gupta et al., 1992). High altitude yaks have also successfully adapted to chronic hypoxia. Like native Himalayans, their small pulmonary arteries are not significantly hypertrophied. However, their pulmonary arterial endothelial cells are much longer, wider, and rounder in appearance than those of domestic cows (their distant cousins) (Durmowicz et al., 1993). Therefore, the mechanisms underlying the adaptive and compensatory responses of the human body to chronic hypoxia are complex and may involve modifications of both pulmonary vascular and endothelial cell function and structure.

Hypoxia-induced PAH can be regarded as somewhat of a channelopathy, whereby deregulated transmembrane ion flux contributes to both HPV and increased PASMC proliferation. HPV is initiated, at least in part, by the membrane depolarization of PASMC due to decreased activity of K^+ channels. K^+ channel dysfunction is indeed associated with HPV and inhibited PASMC apoptosis, whereas Ca^{2+} channel upregulation is also associated with HPV and enhanced PASMC proliferation. This mini-review focuses on the roles played by plasma membrane K^+ and Ca^{2+} channels in the pulmonary artery physiological response to sustained hypoxia.

Role of K⁺ channels

K⁺ is the most abundant intracellular cation (~150 mM in the cytosol vs. ~5 mM in the extracellular space) in most excitable cells, including vascular smooth muscle and cardiac myocytes. Because of this concentration gradient and the positively charged membrane interior, K⁺ ions are passively carried out of the cells according to their electrochemical gradient (Nelson et al., 1995). The resting membrane potential (E_m) in vascular smooth muscle cells is relatively less negative than many excitable cells (e.g., neurons and cardiomyocytes), ranging between -40 and -60 mV, which nears the reversal potential ($E_K \approx -80$ mV) for K⁺. It is generally agreed upon that transmembrane K⁺ permeation through K⁺ channels activity regulate E_m in vascular smooth muscle cells (Nelson et al., 1995). Although multiple types of K⁺ channels have been identified in the pulmonary vasculature, much interest has been placed on the role of voltage-gated K⁺ (Kv) channels in controlling not only E_m (Yuan, 1995; Yuan et al., 1998c; Olschewski et al., 2002), but also the consequent changes in pulmonary vascular tone (Doi et al., 2000; Platoshyn et al., 2001) and PASMC proliferation (Platoshyn et al., 2000; Krick et al., 2001a; Krick et al., 2001b), as described below.

Acute hypoxia decreases K⁺ channel activity, causes membrane depolarization, and induces HPV by mediating Ca²⁺ influx in PASMC

Pulmonary vasoconstriction during acute hypoxia is primarily caused by an increase in cytosolic free Ca²⁺ concentration ($[Ca^{2+}]_{cyt}$) in PASMC. In most cells, Ca²⁺ influx via voltage-dependent Ca²⁺ channels (VDCC) is a major contributor to the increase in $[Ca^{2+}]_{cyt}$ (Post et al., 1995). VDCC inhibition by verapamil attenuates HPV (McMurtry et al., 1976; Yuan et al., 1990), proving that Ca²⁺ influx through VDCC is important for HPV. However, Ca²⁺ influx via voltage-independent Ca²⁺ channels (Post et al., 1995; Robertson et al., 2000) and Ca²⁺ release from the sarcoplasmic reticulum (Jabr et al., 1997; Dipp et al., 2001) are also important sources of elevated $[Ca^{2+}]_{cyt}$ as it relates to the development and maintenance of pulmonary vasoconstriction.

PASMC exposed to hypoxia exhibit significant E_m depolarization and increased $[Ca^{2+}]_{cyt}$ (Yuan et al., 1993). Enhanced $[Ca^{2+}]_{cyt}$ can be mimicked by blockade of Kv channels with 4-aminopyridine (Platoshyn et al., 2000), suggesting that hypoxia-induced increase in $[Ca^{2+}]_{cyt}$ is due to E_m depolarization as a result of decreased Kv channel activity. Indeed, whole-cell Kv currents ($I_{K(V)}$) are decreased in both chronically (Smirnov et al., 1994; Wang et al., 1997; Shimoda et al., 1999; Platoshyn et al., 2001; Michelakis et al., 2002; Pozeg et al., 2003; Hong et al., 2004a) and acutely hypoxic (Yuan et al., 1993; Yuan et al., 1994; Post et al., 1995; Olschewski et al., 2002) PASMC. Therefore, Kv channel inhibition in hypoxic PASMC results in membrane depolarization, activating VDCC, enhancing Ca²⁺ influx, increasing $[Ca^{2+}]_{cyt}$, and then inducing pulmonary vasoconstriction (Fig. 1). Hypoxia-induced inhibition of $I_{K(V)}$ and vasoconstriction are unique to the pulmonary vasculature; hypoxia negligibly affects $I_{K(V)}$ in systemic artery smooth muscle cells and does not cause systemic vasoconstriction (Yuan et al., 1990; Yuan et al., 1993; Platoshyn et al., 2001).

While much of the focus has been on the role of Kv channels in HPV, other K⁺ channels also are affected by hypoxia. The examples that follow are from acute hypoxia studies; however, with further investigation, it may be possible to extrapolate the findings to chronic hypoxic studies. Two-pore domain K⁺ channels (K_T) are voltage-independent, constitutively active channels whose decreased activity has been linked to PASMC depolarization and increased $[Ca^{2+}]_{cyt}$ (Gurney et al., 2002). An oxygen- and pH-sensitive TASK-1 K_T channel isoform may be involved in HPV (Gurney et al., 2002). The role of Ca²⁺-sensitive voltage-dependent K⁺ (K_{Ca}) channels is still unclear. Acute hypoxia has been demonstrated to attenuate large-conductance K_{Ca} channel activity in PASMC (Post et al., 1995), while acute hypoxia-mediated Ca²⁺ release from sarcoplasmic reticulum may enhance the K_{Ca} channel activity (Bonnet et

al., 1994) in PASM. Whatever the case, it is apparent that hypoxic regulation of K_{Ca} channels occurs via the release or production of an intermediate factor during hypoxia (Lee et al., 1994; Post et al., 1995).

Chronic hypoxia downregulates K^+ channel expression in PASM

As mentioned earlier, both acute and chronic hypoxia decreases $I_{K(V)}$, and E_m is partially determined by the amplitude of $I_{K(V)}$ in PASM. $I_{K(V)}$ represents the ensemble of currents generated by all the Kv channels in the cell membrane and varies based on the number of channels (N), the unitary current amplitude of each channel ($i_{K(V)}$), and the probability that the channels are in the open state (P_{open}), according to the following formula: $I_{K(V)} = N \times i_{K(V)} \times P_{open}$. Therefore, the decrease in $I_{K(V)}$ produced by chronic hypoxia can be due to decreased $i_{K(V)}$, P_{open} , or N . The number of channels, which is regulated by expression levels of Kv channel α and β subunits, is particularly important in determining $I_{K(V)}$ amplitude. There is evidence that chronic hypoxia downregulates mRNA and protein expression of Kv channel α subunits in PASM (Wang et al., 1997; Platoshyn et al., 2001; Michelakis et al., 2002; Hong et al., 2004a) (Fig. 2). The hypoxia-induced downregulation of Kv channel α subunit expression decreases the number of functional Kv channels, paralleling the decrease in $I_{K(V)}$ observed in similar models. Surprisingly, Kv channel β subunit expression does not appear to be altered by sustained hypoxic exposure. Hypoxia-mediated decrease in $I_{K(V)}$ and downregulation of Kv channel α subunit expression do not occur in systemic artery smooth muscle cells (Platoshyn et al., 2001), suggesting that the inhibitory effect of hypoxia on Kv channel function and expression is specific to the pulmonary vasculature.

The Kv channel α subunits that are downregulated in chronically hypoxic PASM include: Kv1.1, Kv1.2, Kv1.5, and Kv2.1 (Wang et al., 1997; Platoshyn et al., 2001; Michelakis et al., 2002; Pozeg et al., 2003; Hong et al., 2004a). These α subunits are also believed to participate in forming homo- or hetero-tetrameric channels (e.g., Kv1.2/Kv1.5, Kv2.1/Kv9.3, Kv1.2/Kv β 1.1, and Kv1.5/Kv β 1.2) that are sensitive to acute hypoxia (Patel et al., 1997; Hulme et al., 1999; Osipenko et al., 2000). The mechanisms involved in acute hypoxia-mediated inhibition of Kv channel function may be different from those in chronic hypoxia-mediated downregulation of Kv channel α subunit expression. However, changes in ROS and RNS production, glycolysis and oxidative phosphorylation, and cytochrome P450 and NADPH oxidase activities may contribute to the functional and transcriptional regulation of Kv channels in PASM (Archer et al., 1993; Lee et al., 1994; Yuan et al., 1994; Olschewski et al., 2004). Nevertheless, since *in vivo* gene transfection of Kv1.5 into CH rats restores HPV (Pozeg et al., 2003), it is apparent that Kv downregulation is etiologically associated with hypoxia-induced pulmonary hypertension, not just a secondary effect due to the rise in PAP.

Transcriptional regulation of Kv channel expression

Downregulated K^+ channel expression in chronically hypoxic PASM suggests that gene transcription (Fig. 2) is altered. Not surprisingly, numerous transcription factors are modulated by changes in PO_2 , including activating protein 1 (AP-1), hypoxia-inducible factor1 (HIF-1), and nuclear factor- κ B (NF- κ B) (Semenza, 2000). The latter play important roles in pulmonary disease by influencing mitogenesis, cell proliferation and apoptosis, vascular tone, and inflammation. The promoters of human Kv channel α (e.g., Kv1.5) and β subunit genes include numerous binding sequences for various transcription factors (e.g., c-Jun, NF- κ B, C-Myb, CREB, c/EBP, γ -IRE) (Remillard and Yuan, unpublished observations). Chronic hypoxia may alter the activity and/or production of these transcription factors via changes in cellular redox status, mitochondrial function and metabolism and NADPH oxidase activity, and directly modulate transcription of Kv channel genes in PASM. Chronic hypoxia may also exert its indirect regulatory effect on Kv channel gene transcription and expression via HIF-1.

HIF-1 is a multipotent transcription factor which is not only activated by hypoxia, but whose activity is integral to many signalling pathways involved in HPV and CH-induced pulmonary vascular remodelling. Partial HIF-1 α deficiency (*HIF1*^{-/+} mice) can significantly delay the development of pulmonary vascular remodeling, pulmonary hypertension, and right ventricular hypertrophy during chronic hypoxic exposure (Yu et al., 1999). In a subsequent study, Brusselmanns et al. showed that mice partially deficient in HIF-2 α (*HIF2*^{-/+}), a HIF-1 α homologue, were totally protected against pulmonary hypertension and right ventricular hypertrophy (Brusselmanns et al., 2003). In addition, endothelin-1 (ET-1) expression was enhanced threefold in the lungs of *HIF2*^{-/+} mice, suggesting that HIF-2 α may upregulate ET-1. A previous study showed that ET-1 causes pulmonary vasoconstriction and stimulates PASMC proliferation by, at least in part, inhibiting Kv channel function and expression (Shimoda et al., 2001). While there is no indication that HIF-2 α affects Kv channel function, we can hypothesize that the hypoxia-mediated production of ET-1 via HIF may serve as an important mechanism in downregulating Kv channel α subunit expression in PASMC. Furthermore, like ET-1, the genes encoding for vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and erythropoietin (EPO) contain HIF-1 hypoxia-response element (HRE) binding sequences. Therefore, hypoxia-mediated production of VEGF, PDGF, EPO and ET-1 via HIF-1 binding could, theoretically, inhibit Kv activity to enhance pulmonary vasoconstriction and stimulate PASMC proliferation, thereby causing vascular remodeling.

c-Jun, a transcription factor belongs to the AP-1 family, may be an important intermediate in hypoxia-mediated gene regulation based on its ability to sense changes in cytoplasmic redox potential and [Ca²⁺]_{cyt} (Abate et al., 1990). In normoxic PASMC, overexpression of c-Jun decreases *I*_{K(V)} and downregulates α and upregulates β Kv subunits' expression (Yu et al., 2001). The c-Jun-mediated downregulation of Kv channel α subunit expression and decreased Kv channel activity mimic the effects of sustained hypoxia. Because of the divergent effects on Kv channel α and β subunits, c-Jun may modulate their expression differentially, as has been suggested for the *hsl* gene and its regulatory β subunit (Dhulipala et al., 1999). The downregulated α subunit and upregulated β subunits in PASMC during chronic hypoxia would decrease *I*_{K(V)} as a result of decreased current amplitude and accelerated current inactivation (Yu et al., 2001).

Finally, silencer binding elements (regulatory DNA elements that control cell-specific gene expression) may play a role in modulating Kv channel gene expression. Mori et al. (Mori et al., 1995) and Valverde et al. (Valverde et al., 1999) reported the existence of a K⁺ repressor element (KRE) binding site in the promoter of the cardiac Kv1.5 gene. A dinucleotide repetitive element within the KRE site is required for silencer activity; its deletion promotes Kv1.5 channel expression (Mori et al., 1995). A KRE binding factor (KBF) abolishes this silencing effect, enhancing Kv1.5 expression. While neither the KRE site nor the KBF have yet to be identified in the promoter of Kv channels in PASMC, it is intriguing to speculate what its putative role could be in regulating Kv channel expression in the pulmonary vascular as well as pulmonary vasoconstriction and medial hypertrophy during chronic hypoxia.

K⁺ efflux and PASMC apoptosis: role in vascular remodeling

As indicated earlier, vasoconstriction is but one component of the pulmonary arterial response to chronic hypoxia. Pulmonary medial hypertrophy occurs as a result of the combination of unchecked PASMC proliferation and attenuated PASMC apoptosis. Inhibition of K⁺ channel activity and/or Kv gene downregulation, as seen in during chronic hypoxia and in IPAH patients (Yuan et al., 1998a; Yuan et al., 1998b), play dual roles in modulating PASMC proliferation, both by promoting proliferation and by attenuating apoptosis.

First, the membrane depolarization associated with decreased $I_{K(V)}$ causes Ca^{2+} influx and Ca^{2+} release from intracellular stores. In addition to its prominent role in modulating vascular tone, Ca^{2+} is also required for cell proliferation (Berridge et al., 2003) and gene expression (Hardingham et al., 1997). Therefore, newly introduced intracellular Ca^{2+} is available to trigger transcription of proliferative genes and to allow progression of the cell cycle. We have previously demonstrated the dependence of proliferation on Ca^{2+} influx in rat PASM, where buffering of extracellular Ca^{2+} , i.e., prevention of Ca^{2+} influx, attenuates proliferation (Platoshyn et al., 2000).

Secondly, K^+ efflux is a prerequisite for apoptotic cell shrinkage, an early hallmark of apoptosis, and high intracellular K^+ inhibits cytoplasmic caspases. Numerous studies indicate that blockade of K^+ efflux through K^+ channels decelerates apoptotic volume decrease (i.e., cell shrinkage), attenuates caspase activation in the cytoplasm, inhibits cytochrome *c* release, and decreases DNA fragmentation induced by apoptosis inducers (Remillard et al., 2004). K_{Ca} and K_v channels appear to be the prime mediators of these effects in PASM (Krick et al., 2001a; Krick et al., 2001b; Platoshyn et al., 2002), while K_T and K_{ATP} (ATP-sensitive) channels also have been implicated in other cell types (Yu et al., 1997; Trimarchi et al., 2002). To summarize, enhanced K^+ efflux promotes apoptosis by accelerating apoptotic cell shrinkage and removing suppressive effect of cytoplasmic K^+ on caspases and nucleases (Fig. 3). Hypoxia-mediated decrease in $I_{K(V)}$ in PASM *a*) stimulates cell proliferation by depolarization-induced increase in $[Ca^{2+}]_{cyt}$ and *b*) attenuates cell apoptosis by decelerating apoptotic cell shrinkage and by inhibiting cytoplasmic caspases. The proliferative and antiapoptotic effects of hypoxia on PASM via decreased K^+ channel activity then contribute to the development of pulmonary vascular medial hypertrophy and pulmonary hypertension (Fig. 2). This suggestion is supported by recent reports showing that *a*) overexpression of $Kv1.5$ in normoxic PASM increases $I_{K(V)}$, accelerates apoptotic volume decrease, and enhances apoptosis (Brevnova et al., 2004), and *b*) *in vivo* gene transfer of $Kv1.5$ causes regression of pulmonary vascular remodeling in chronically hypoxic rats (Pozeg et al., 2003). Therefore, as for HPV, K_v downregulation also is etiologically associated with the vascular remodelling caused by chronic hypoxia-induced pulmonary hypertension.

Role of Ca^{2+} channels

Modes of Ca^{2+} influx

It is now apparent that Ca^{2+} influx across the plasma membrane plays an important role in the pulmonary vasculature's response to sustained hypoxia as seen at high altitude. Two primary modes of Ca^{2+} influx exist in vascular smooth muscle cells: voltage-dependent (VDCC) and -independent Ca^{2+} channels. In the pulmonary vasculature, Ca^{2+} influx through VDCC is responsible for the sustained PASM contraction associated with HPV. O_2 -sensitive VDCC have been identified in cells that modulate the pulmonary hypoxic response, such as carotid (glomus cells) (Montoro et al., 1996) and PASM (Franco-Obregon et al., 1996). However, it is unclear precisely what their role is in the pulmonary pressor response to sustained hypoxia.

The physiological role of voltage-independent Ca^{2+} channels in PASM and endothelial cells has become a hot topic in the past few years. These channels activate independently of changes in E_m . Rather, their opening requires either stimulation of membrane receptors (receptor-operated Ca^{2+} channels, ROC) or depletion of the sarcoplasmic reticulum Ca^{2+} store (store-operated Ca^{2+} channels, SOC). In PASM, a nifedipine-insensitive Ca^{2+} current is activated by hypoxia (acute and chronic), with its activity blocked by Ni^{2+} , La^{3+} and SK&F 96365, inhibitors of ROC and SOC (Robertson et al., 2000; Kang et al., 2003; Wang et al., 2004b). Therefore, Ca^{2+} influx through ROC and/or SOC also appears to play a prominent role in elevating $[Ca^{2+}]_{cyt}$ and mediating pulmonary vasoconstriction under hypoxic conditions. The Ca^{2+} influx due to store depletion-mediated activation of SOC, namely capacitative Ca^{2+} entry

(CCE), has also been implicated in the pulmonary remodeling response to sustained hypoxia (see below).

Cell-specific TRP expression in the pulmonary vasculature

Transient receptor potential (TRP) genes are believed to encode for ROC and SOC (Parekh et al., 1997). Functional ROC and SOC are formed by homomeric or heteromeric tetramers composed of different TRP channel subunits, especially the canonical-type TRP (TRPC) subunits. Expression of TRPC channels exhibits some cell-specificity within the pulmonary vasculature. PASM from normal subjects and normotensive rats express high levels of TRPC1, TRPC4, and TRPC6 (Golovina et al., 2001; Lin et al., 2004; Wang et al., 2004a; Yu et al., 2004). On the other hand, pulmonary artery endothelial cells (PAEC) express high levels of TRPC1, TRPC3, and TRPC4 (Kamouchi et al., 1999; Fantozzi et al., 2003; Yu et al., 2004). Therefore, while TRPC1 and TRPC4 are ubiquitously expressed, TRPC3 and TRPC6 seem to be dominant in PAEC and PASM, respectively (Yu et al., 2004). As stated earlier, native ROC and SOC are likely heterotetrameric channels formed by different TRPC subunits. This is supported by observations that TRPC1 and TRPC4 or TRPC5 can form a functional channel that is activated by receptor activation and store depletion (Strübing et al., 2001), while TRPC3 and TRPC6 heterotetramers form functional ROC in systemic arterial smooth muscle cells (Hofmann et al., 2002) and functional SOC in PASM (Yu et al., 2004). Therefore, whether the functional channel is ROC or SOC appears to be determined by the composition of TRPC subunits and/or co-localization of the channels with receptors in the plasma membrane and sarcoplasmic reticulum.

Voltage-independent Ca^{2+} influx in PASM

Agonist-mediated pulmonary vasoconstriction involves Ca^{2+} influx through VDCC, ROC, and SOC). For example, phenylephrine-induced pulmonary vasoconstriction is partially due to CCE via SOC and Ca^{2+} influx via ROC (McDaniel et al., 2001). In addition, overexpression of TRPC1 in isolated pulmonary arterial rings enhances CCE-mediated contraction relative to rings transfected with the empty vector (Kunichika et al., 2004). These observations suggest that upregulated TRPC channels in PASM would enhance pulmonary vasoconstriction by promoting receptor-mediated and/or store depletion-mediated Ca^{2+} influx.

In addition to augmenting pulmonary vasoconstriction, upregulation of TRPC channels and enhancement of CCE also contribute to stimulating PASM growth in the presence of growth factors. In proliferating PASM (from normal subjects or normotensive rats), the mRNA and protein expression of TRPC channels (e.g., TRPC1, TRPC3, and TRPC6) is upregulated and the amplitude of SOC currents (I_{SOC}) and CCE is augmented, in comparison to growth-arrested PASM (Golovina et al., 2001). In PASM from patients with idiopathic pulmonary arterial hypertension (IPAH), expression levels of TRPC3 and TRPC6 are much higher than in PASM from normal subjects and normotensive patients (Yu et al., 2004). Inhibition of TRPC1 and TRPC6 with antisense oligonucleotides and siRNA decreases I_{SOC} and CCE, and attenuates PASM proliferation (Sweeney et al., 2002). These observations provide compelling evidence that upregulated TRPC channels are involved in PASM proliferation, whereas downregulation of TRPC channels inhibits PASM proliferation.

Experiments *in vivo* and *in vitro* have demonstrated that chronic hypoxia upregulates TRPC channels (e.g., TRPC1 and TRPC6) and enhances CCE in PASM (Hong et al., 2004b; Lin et al., 2004; Ng et al., 2004; Wang et al., 2004b; Wang et al., 2005) (Fig. 4A and B). The resultant elevation in $[\text{Ca}^{2+}]_{\text{cyt}}$ as a result of receptor- or store depletion-mediated Ca^{2+} influx would be an additional mechanism for hypoxia-mediated pulmonary vasoconstriction and PASM hypertrophy and hyperplasia. Indeed, many of the latter studies indicate that hypoxia-induced CCE via SOC enhances pulmonary artery tension (Hong et al., 2004b; Lin et al., 2004; Wang

et al., 2004b), thereby contributing to HPV and hypoxia-induced PAH (Fig. 4). That enhanced CCE and SOC activity is also associated with upregulated TRPC channel expression in PASM (Hong et al., 2004b; Lin et al., 2004; Wang et al., 2004b) supports prior indications that TRPC genes encode for SOC channels.

Voltage-independent Ca^{2+} influx in PAEC

While PAEC do not contribute directly to either the vasoconstriction or the medial hypertrophy associated with hypoxia-mediated PAH, endothelium-derived constricting/relaxing and growth factors are extremely important in the regulation of pulmonary vascular tone and PASM proliferation. Indeed, hypoxia triggers endothelial production and secretion of many vasoactive substances and mitogens that influence PASM, such as ET-1, thromboxane A_2 , prostacyclin, nitric oxide, matrix metalloproteins, PDGF, and VEGF (Faller, 1999). In addition, as discussed earlier, hypoxia also enhances the DNA binding activity of the transcription factors (e.g., AP-1, HIF-1, NF- κ B) that modulate expression of ET-1, PDGF, and VEGF (Faller, 1999; Semenza, 2000).

The roles of Ca^{2+} influx via TRPC channels in endothelial cells are diverse. Activation of TRPC1 and TRPC4 channels in PAEC promotes cell shape changes, increases vascular permeability and influences angiogenesis (Moore et al., 1998b). In systemic (e.g., aortic) endothelial cells, however, TRPC4 deficiency appears to impair agonist-induced vasorelaxation (Freichel et al., 2001). These observations suggest that Ca^{2+} influx via TRPC channels in pulmonary and systemic endothelial cells may serve different functions: it causes PAEC contraction and stimulates PAEC proliferation, whereas induces synthesis and release of relaxing factors (e.g., nitric oxide) from systemic endothelial cells. Under normoxic conditions, a rise in $[\text{Ca}^{2+}]_{\text{cyt}}$ in PAEC would stimulate production of nitric oxide (NO) as a result of Ca^{2+} -mediated activation of O_2 -dependent endothelial nitric oxide synthase (eNOS). Since eNOS activity is inhibited during persistent hypoxia, the increased $[\text{Ca}^{2+}]_{\text{cyt}}$ in PAEC would more likely cause PAEC contraction and/or stimulate synthesis of growth factors.

In human PAEC, chronic hypoxia significantly upregulates the mRNA and protein expression of TRPC4, augments the activity of SOC, enhances the amplitude of CCE, raises $[\text{Ca}^{2+}]_{\text{cyt}}$, and increases the AP-1 DNA binding activity (Fig. 4C-E) (Fantozzi et al., 2003). Many AP-1 responsive genes encode for endothelium-derived mitogens (e.g., ET-1, PDGF and VEGF) that can be increased in PAEC and released to intercellular space between PAEC and PASM. The endothelium-derived vasoconstrictors and mitogens may then exert their vasoconstrictive and mitogenic effects on PASM via a paracrine mechanism, thereby causing pulmonary vasoconstriction and mediating vascular medial hypertrophy. Enhanced TRPC expression in PAEC following hypoxic exposure may therefore have a significant effect on the development of chronic hypoxia-mediated PAH.

Conclusion

Sustained alveolar hypoxia such as that experienced by high-altitude residents has a severe physiological impact on the pulmonary vasculature. The haemodynamic outcome (i.e., increased PAP) is due to enhanced pulmonary vasoconstriction and pulmonary vascular remodeling, both of which cause reduction of the vascular lumen and an increase in pulmonary vascular resistance. In the pulmonary vasculature, it is apparent that transmembrane ion flux plays an important role in controlling the cellular processes that underlie both vasoconstriction and medial hypertrophy and hyperplasia during hypoxic exposure. From our discussion, it is clear that the flow of K^+ and Ca^{2+} through their respective ion channels modulates these processes by controlling cellular volume, membrane potential, $[\text{Ca}^{2+}]_{\text{cyt}}$, gene transcription, apoptosis, and cell cycle progression. Precisely how K^+ and Ca^{2+} channels 'sense' changes in O_2 tension or how their activity and expression somehow "adapt" at higher altitudes is unclear.

These are the million dollar questions that pulmonary physiologists wish to answer. However, it is becoming increasingly apparent that ion channels themselves are likely not the actual O₂ sensors. Even more ambiguous is whether the pulmonary O₂ sensor actually resides in PASMC, or whether it is located in PAEC, or carotid and neuroepithelial bodies associated with the pulmonary vasculature. Needless to say, there is more work to be done.

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Glossary

Ca²⁺, Ca²⁺ ion
 [Ca²⁺]_{cyt}, cytosolic Ca²⁺ concentration
 CH, chronic hypoxia
 CCE, capacitative Ca²⁺ entry
 HPV, hypoxic pulmonary vasoconstriction
 K⁺, potassium ion
 Kv, voltage-dependent K⁺ channel
 I_{K(V)}, Kv current
 PAEC, pulmonary artery endothelial cell
 PAH, pulmonary arterial hypertension
 PASMC, pulmonary artery smooth muscle cell
 ROC, receptor-operated Ca²⁺ channel
 SOC, store-operated Ca²⁺ channel
 TRP, transient receptor potential
 VDCC, voltage-dependent Ca²⁺ channel

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Figure 1.

Geometric and morphological changes induced by chronic hypoxia. (A) Diagram depicting hypoxia-induced vasoconstriction and vascular remodeling in pulmonary arteries (PA). Both vasoconstriction (B) and medial hypertrophy (C) are clearly demonstrated in an X-ray image (B) and lung histological preparations (C). Images in B and C are reproduced with permission from (Dawson, 2004) and (Michelakis et al., 2002), respectively.

Figure 2.

Effect of sustained hypoxia on K^+ channel gene transcription (A), gene expression (B), K_v currents (C), membrane potential (D), resting $[Ca^{2+}]_{cyt}$ (E), and concomitant effects on both pulmonary vascular tone (F) and remodeling (G). In panels B-E inclusively, effects of hypoxia are shown in the unboxed images on the right. TF, transcription factor.

Figure 3.

[K⁺]_{cyt} flux influences apoptosis. Normally, the high level of cytoplasmic K⁺ ([K⁺]_{cyt}) contributes to maintaining cell volume and suppresses (–) cytoplasmic caspase activity, thereby inhibiting the onset of apoptotic cell shrinkage and the apoptotic cascade. Opening or upregulation of K⁺ channels would accelerate (+) apoptotic volume decrease and enhance apoptosis by increasing K⁺ efflux and loss.

Figure 4.

Effect of chronic hypoxia on TRPC channel expression and function in PASMC (A-B) and PAEC (C-E) in the modulation of pulmonary vascular tone and medial hypertrophy. Panels A and C describe the effects of sustained hypoxia on TRPC expression in PASMC and PAEC, respectively. Effects of hypoxia on I_{SOC} and CCE are shown in panels B and D for both preparations. In PASMC, enhanced TRPC expression and CCE lead to increased PASMC contraction and proliferation. In PAEC, increased CCE enhances Ca^{2+} -dependent AP-1 binding activity (E), thereby promoting the transcription of mitogens and growth factors such as ET-1 and PDGF, which stimulate PASMC contraction and proliferation via paracrine interactions with their respective G protein-coupled receptor (GPCR) and receptor tyrosine kinase (RTK) on PASMC membranes. Traces in panels B and D are reproduced with permission from (Lin et al., 2004) and (Fantozzi et al., 2003), respectively.