Invited Review for the 2009 Hirosi Kuriyama Award

Patho-, physiological roles of voltage-dependent K+ channels in pulmonary arterial smooth muscle cells

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Abstract

In this review, we demonstrate the basic properties, modulation of, and pathological changes in voltage-dependent K+ (Kv) channels that are expressed in pulmonary arterial smooth muscle cells (PASMCs). Pulmonary Kv channels are thought to play a crucial role in the maintenance of resting membrane potentials, and therefore the vascular tone of the pulmonary arteries. Although the molecular identity of pulmonary Kv channels is not clear, Kv1.1, Kv1.2, Kv1.5, Kv2.1, Kv9.3, and Kv3.1 subtypes are expressed in PASMCs. In addition, resistant PASMCs contain greater amount of Kv channels as compared to conduit PASMCs. This heterogenous expression of Kv channels is consistent with regional differences in the contractile response to hypoxia. Similar to other K+ channels, pulmonary Kv channels can also be modulated by several vasoconstrictors concomitant with the activation of protein kinase C (PKC). Alterations in Kv channel function have several additional and interrelated consequences, including the regulation of cell proliferation and apoptosis, which ultimately lead to pulmonary vascular remodeling. Increased pulmonary vasoconstriction in pulmonary arterial hypertension is attributable to decreased expression and activity of Kv channels in smooth muscle cells. Kv channels play a central role in the maintenance of cellular homeostasis and ion channels, and consequential signaling cascades. Therefore, Kv channels are potential therapeutic targets for the treatment of pulmonary vascular disease.

Key words: hypoxic pulmonary vasoconstriction, pulmonary arterial hypertension, pulmonary arterial smooth muscle cells, voltage-dependent K+ channels

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Introduction

The pulmonary circulation is maintained as a high-flow, low-pressure, and low-resistance system during normal ventilation and it supplies oxygenated blood to the rest of the body. Numerous studies have suggested that defects in pulmonary arterial smooth muscle cell (PASMC). K+ channels are involved in the development of pulmonary disease (e.g., primary pulmonary hypertension) (Yuan et al., 1998a, 1998b). Similar to other vessels, PASMCs express several distinct types of K+ channels, including ATP-sensitive K+ (KATP), Ca2+-activated K+ (BKCa), and voltage-dependent K+ (Kv) channels (Nelson, 1993; Yuan, 1995; Peng et al., 1996). Among the K+ channels, Kv channels are regarded as major regulators of membrane potential, since inhibition of Kv channels, but not BKCa or KATP channels, induces membrane depolarization and leads to an increase in intracellular Ca2+ concentrations (Sweeney and Yuan, 2000; Shimoda et al., 2001; Mandegar and Yuan, 2002). Furthermore, evidence suggests that acute and chronic hypoxic responses and the development of pulmonary hypertension are closely related to alterations in Kv channel function and/or expression (Wang et al., 1997; Platoshyn et al., 2001; Mandegar and Yuan, 2002).

To better understand Kv channels in PASMCs, we review their basic properties, their modulation by protein kinase C (PKC), and the pathological alterations of Kv channels in PASMCs.

Basic properties of Kv channels in PASMCs

Physiological roles of Kv channels in PASMCs

As described in the introduction, several classes of K+ channels have been identified in PASMCs based on their electrophysiological and pharmacological properties. Among these channels, the Kv channels are most likely to regulate resting membrane potential, and therefore basal vascular tone (Post et al., 1995; Yuan, 1995; Evans et al., 1996; Yuan et al., 1998c). In fact, application of a Kv channel inhibitor (4-aminopyridine; 4-AP), but not BKCa or KATP channel inhibitors, decreases Kv currents and causes membrane depolarization, which opens L-type Ca2+ channels and subsequently triggers pulmonary vasoconstriction (Platoshyn et al., 2000).

Increased cytosolic Ca2+ caused by blockade of Kv channels promotes cell proliferation. A rapid increase in nuclear Ca2+ concentration is concomitant with a rise in cytosolic Ca2+, which accelerates cell proliferation via modulation of the cell cycle (Allbritton et al., 1994; Means, 1994; Hardingham et al., 1997; Mandegar and Yuan, 2002). Cytosolic Ca2+ propels cellular proliferation by phosphorylation of proliferation-related signal transduction proteins (Chao et al., 1992; Berridge, 1993). Furthermore, a contraction induced by a rise in cytosolic Ca2+ stimulates the pulmonary arterial cellular overgrowth, which leads to pulmonary hypertrophy (Hishikawa et al., 1994; Kolpakov et al., 1995). Therefore, increased intracellular (cytosolic and nuclear) Ca2+ caused by blockade of Kv channels promotes cellular proliferation due to increased in gene expression, phosphorylation, and contraction in PASMCs, which contribute to vascular remodeling.

A decrease in intracellular K+ through Kv channels is believed to initiate apoptosis.
Therefore, maintenance of high concentrations of intracellular K$^+$ via decreased Kv channel activity is required for the suppression of caspases and nucleases, which are regarded as mediators of apoptosis (Bortner et al., 1997; Hughes et al., 1997; Lang et al., 1998; Mandegar and Yuan, 2002). This process also facilitates vascular remodeling (Fig. 1).

Therefore, drug targeting of Kv channels, which inhibit intracellular Ca$^{2+}$ and cause cellular proliferation and augmentation of apoptosis in PASMCs, could provide a potential therapy for pulmonary diseases.

**Molecular identification of Kv channels in PASMCs**

Kv channels consist of pore-forming α subunits, which are composed of six transmembrane domains (S1–S6) and ancillary β subunits (Korovkina and England, 2002; Ko et al., 2008). Each α subunit can be associated with β subunits, which determines the characteristics of the channel (Bahring et al., 2001; Ko et al., 2008).

Although numerous studies have examined the electrophysiological and pharmacological properties of Kv channels in PASMCs, few have performed molecular identification of Kv channels using RT-PCR and immunoblotting. With respect to the α subunits, most studies examining the molecular subtypes of Kv channels in PASMCs report the expression of Kv1.1, Kv1.2, Kv1.5, Kv2.1, and Kv9.3 (Archer et al., 1998, 2004; Yuan et al., 1998c; Osipenko et al., 2000; Coppock et al., 2001; Davies and Kozlowski, 2001; Platoshyn et al., 2001). Expression

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**Fig. 1.** Schematic diagram for the onset of pulmonary diseases. [Ca$^{2+}$]$_{cyt}$, cytosolic Ca$^{2+}$; [Ca$^{2+}$]$_{n}$, nuclear Ca$^{2+}$; [K$^+$]$_{i}$, intracellular K$^+$. 

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levels of Kv1.3, Kv1.4, and Kv1.6 are relatively controversial in PASMCs. Archer et al. (1998) proposed the presence of Kv1.1, Kv1.2, Kv1.3, Kv1.5, Kv1.6, and Kv2.1, but they failed to detect Kv1.4. At about the same time, Yuan et al. (1998) demonstrated the expression of Kv1.1, Kv1.2, Kv1.4, Kv1.5, Kv1.6, Kv2.1, and Kv9.3, but not Kv1.3. A few years later, expression of the Kv3.1 subtype was reported by other groups (Osipenko et al., 2000; Coppock et al., 2001; Archer et al., 2004). Although other subtypes (Kv1.7, Kv4.1, and Kv9.2) have also been identified (Davies and Lozlowski, 2001), Kv1.1, Kv1.2, Kv1.5, Kv2.1, Kv9.3, and additionally Kv3.1 subtypes are expressed in PASMCs, and one (or a co-assembly) of these types may act as oxygen sensors.

In PASMCs, combinations of β subunits with α subunits increase the diversity of Kv channel function, including inactivation and deactivation kinetics, and voltage sensitivity (Morales et al., 1996; Uebele et al., 1998). To date, four types of β subunits (Kvβ1–4) have been identified. Among these types, Kvβ1, Kvβ2, and Kvβ3 subunits are expressed in PASMCs (Standen and Quayle, 1998; Yuan et al., 1998c). Although the exact function of β subunits is not known, β subunits play a crucial role as a redox sensor and in hypoxic adaptation (Perez-Garcia et al., 2000; Coppock et al., 2001).

**Regional diversity of Kv channels in PASMCs**

Diversity in K⁺ channel distribution is well documented between conduit and resistance arteries, which contribute to differences in the pulmonary arterial response to hypoxia with respect to pharmacological and electrophysiological properties (Archer et al., 1996). The predominant site of hypoxic pulmonary vasoconstriction (HPV) in the lung appears to be in the small pulmonary resistance arteries (< 200–300 μm diameter) (Shirai et al., 1986; Kato and Staub, 1996). These regional differences between conduit and resistance PASMCs may reflect distributional differences in Kv and BKCa channels (Archer et al., 1996, 2004; McCulloch et al., 2000; Smirnov et al., 2002; Bonnet and Archer, 2007). For example, conduit PASMCs predominantly express BKCa channels, which results in more sensitive inhibition by TEA or the specific BKCa channel blocker, charybdoctoxin (Garcia et al., 1991). In contrast, resistance PASMCs contain greater numbers of Kv channels rather than TEA-sensitive BKCa channels. Therefore, resistance PASMCs are inhibited by low concentrations of 4-AP, but lack sensitivity to TEA or charybdoctoxin blockade (Archer et al., 1996; Smirnov et al., 2002). This heterogenetic channel expression is also consistent with regional differences in the contractile response to hypoxia. Isolated vascular ring experiments support the tenet that resistant pulmonary arteries cause more significant and sustained hypoxic constriction as compared to conduit arteries (Harder et al., 1985). In contrast, conduct vessel hypoxia shows poor vasoconstriction and even vasodilation (Weir and Archer, 1995). At the protein level, resistance arteries are particularly enriched in Kv1.5, a 4-AP sensitive, hypoxia-inhibited channel that is involved in the mechanisms of HPV (Archer et al., 2004). Electrophysiological data show that resistance pulmonary arteries contract more prominently in response to the Kv1.X-specific inhibitor, correolide, irrespective of increased 4-AP sensitivity. In addition, the combination of anti-Kv1.5 and -Kv2.1 cause greater depolarization than either antibody alone in resistance PASMCs (Archer et al., 2004). This is consistent with previous publications that K⁺ efflux through Kv1.X and Kv2.X channels are the primary determinant of the resting membrane.
potential in the resistant PASMCs (Patal et al., 1997; Hogg et al., 2002; Archer et al., 2004). Consistent with the electrophysiological data, protein expression of Kv1.5 are greater in resistance arteries (Archer et al., 2004). The enrichment of resistance PASMCs with \( \text{O}_2 \)-sensitive Kv1.5 and Kv2.1 subtypes accounts for the localization of HPV to this zone of the pulmonary circulation. In a view of these accumulating results, heterogenetic expression of \( K^+ \) channels along the length of the pulmonary arterial diameter leads to functional diversity in hypoxia responses (Archer et al., 1996). The molecular basis for this ionic diversity, however, requires further study.

**Modulation of Kv channels in PASMCs**

**PKC subtypes in PASMCs**

Generally, several protein kinase C (PKC) subtypes including \( \alpha, \beta, \beta_0, \delta, \epsilon, \) and \( \zeta \), have been identified in vascular smooth muscle (Dixon et al., 1994; Lee and Severson, 1994; Ward et al., 2004). These subtypes are classified into three main groups (Howe and Abdel-Latif, 1988): conventional (classic), novel, and atypical PKC. In vascular smooth muscle, \( \alpha \) and \( \beta \) subtypes are included in conventional PKC, which require at least \( \text{Ca}^{2+} \), diacylglycerol, and phosphatidylserine for activation. Novel PKC, including \( \delta \) and \( \epsilon \) subtypes, requires diacylglycerol and phosphatidylserine, but not \( \text{Ca}^{2+} \). Atypical subtypes (\( \zeta \)) do not require \( \text{Ca}^{2+} \) and diacylglycerol, but are instead activated by phosphatidylserine and phosphatidylinositol 3,4,5-triphosphate (PIP3). Similar to other vascular tissues, subtypes PKC-\( \alpha \), \( \beta \), \( \beta_0 \), \( \delta \), \( \epsilon \), and \( \zeta \) are expressed in the pulmonary artery, despite the fact that PKC subtypes are slightly different among the species, vascular beds, and artery sizes (Lee et al., 1999; Dallas and Khalil, 2003). For example, PKC-\( \alpha \), \( \delta \), \( \epsilon \), and \( \zeta \), as well as \( \mu, \upsilon \), and \( \lambda \) subtypes, are expressed in primary cultured cells of canine PASM, but the PKC\( \beta \) subtype is not detectable (Damron et al., 1998). In feline PASM, PKC-\( \alpha \) and \( \delta \) can be detected by immunohistochemistry (De Witt et al., 2001).

**Effects of vasoconstrictors on Kv channel in PASMCs: role of protein kinase C**

Although vasoconstrictors such as angiotensin II, endothelin-1, vasopressin, and thromboxane are widely accepted to inhibit Kv channels by activating phospholipase C (PLC) and PKC in various vascular smooth muscle cells, the subtypes of PKC involved in the regulation of pulmonary Kv channels vary among the vasoconstrictors. For example, serotonin (5-hydroxytryptamine) inhibits Kv channels in rat pulmonary arteries by activating conventional \( \text{Ca}^{2+} \)-dependent PKC subtypes, not PKC\( \zeta \) (Cogolludo et al., 2006). The same group, however, also showed that Kv channel inhibition by thromboxane A2 is mediated by the activation of PKC\( \zeta \) (Cogolludo et al., 2003; Ko et al., 2008). Regarding the inhibitory effects of endothelin-1 on pulmonary Kv channels, both \( \text{Ca}^{2+} \)-dependent and -independent subtypes of PKC are involved (Shimoda et al., 1998) (Fig. 2). In systemic circulation, the involvement of PKC subtypes is different from that of pulmonary ones. For example, endothelin-1 inhibits mesenteric Kv channels mainly by activating of \( \text{Ca}^{2+} \)-independent PKC\( \epsilon \) subtypes (Rainbow et al., 2009). Furthermore, angiotensin II acts via the activation of \( \text{Ca}^{2+} \)-dependent PKC\( \alpha \) subtypes to inhibit the mesenteric Kv channel (Hayabuchi et al., 2001; Rainbow et al., 2009). To date, however,
limited studies on the regulation of pulmonary and systemic arterial Kv channels by specific PKC subtypes make it difficult to understand entire signaling cascades of vasoconstrictor-PKC subtypes-Kv channels; therefore, intensive studies should be performed in the near future.

Pathological alterations in Kv channels

The characteristic increased pulmonary vasoconstriction in pulmonary arterial hypertension (PAH) is attributable to decreased expression and activity of Kv channels in smooth muscle cells (Humbert et al., 2004). Alterations in the function of Kv channels have several additional and interrelated consequences, including the regulation of cell proliferation and apoptosis, which ultimately leads to pulmonary vascular remodeling. Smooth muscle cell Kv channels are inhibited by acute and chronic exposure to hypoxia (Fig. 3). This inhibition leads to cell membrane depolarization, which enhances the rate of calcium influx via voltage-gated channels and causes further vessel constriction. Indeed, many studies have reported reduced Kv channel function in both hypertension and hypertrophy (Martens and Gelband, 1996; Yuan, et al., 1998a; Yuan and Rubin, 2005). A similar phenomenon is observed in persistent pulmonary hypertension of newborn (PPHN) (Konduri et al., 2009).

Hypoxic pulmonary vasoconstriction (HPV) and chronic hypoxic pulmonary hypertension (CH-PHT)

Hypoxic pulmonary vasoconstriction (HPV) is a well documented physiological response to alveolar hypoxia, which acts to maximize oxygenation of the blood by diverting it from poorly oxygenated regions to areas of high oxygen partial pressure. When vasoconstriction of the
Pulmonary diseases and K+-channels

Pulmonary vasculature is sustained, hypoxia-induced vascular remodeling occurs and chronic-hypoxic pulmonary hypertension (CH-PHT) ensues. HPV is a complex multifactorial mechanism for which the exact underlying pathways are yet to be deciphered. Ion channels such as L-type calcium channels, nonspecific cation channels and voltage-dependent potassium channels have been established to have inherent roles in the effector mechanism. Although some evidence exists for Kv channels themselves being O2 sensors, it seems more likely that the prevailing mechanism involves the mitochondria as the O2 sensors and mitochondria-dependent factors such as reactive oxygen species (ROS) acting as mediators.

The effects of hypoxia on the pulmonary vasculature are unique and do not occur in the systemic circulation. For example, hypoxia differentially affects vessel tone in pulmonary and mesenteric arteries, which correlates with hypoxia-induced inhibition of K+ currents in PASMC. This effect is accompanied by an enhancement of the inward Ca2+ current in the pulmonary artery, an effect not observed in MASMCs (Yuan et al., 1990; Yuan et al., 1993).

Fig. 3. Effect of acute hypoxia on Kv current in PASMCs. Kv currents obtained in the control (A), hypoxia (B), and wash out (C) using step pulses from −120 mV to +80 mV in steps of 20 mV. (D) Current-voltage (I-V) relationships of the Kv currents in the control (○) and hypoxia (●), n=5. *P<0.05. (E) Changes of resting membrane potential during hypoxia, n=4.

Delayed rectifier currents that are sensitive to 4-AP are deemed to be important determinants of resting membrane potential (E_m) and critical in the initiation of HPV (Post et al., 1992; Archer et al., 1998). Despite being proposed to be molecular correlates for O2 sensing in PASMCs (López-Barneo, 1994), it is more generally accepted that their function is altered by a factor stimulated by hypoxia, which subsequently causes inhibition of the channel. Post and colleagues were the first to identify K+ channel inhibition as a key event in the mechanisms of HPV via their role in membrane depolarization and subsequent opening of voltage-gated Ca2+ channels and the
promotion Ca²⁺ entry (Post et al., 1992). Indeed, decreased Kv channel currents have been observed in response to acute hypoxia in both rat and mouse PASMCs (Archer et al., 1993, 2001; Yuan et al., 1993; Turner and Kozlowski, 1997; Platsoshyn et al., 2006). Also note that Smirnov et al. (1994) showed that chronic hypoxia (PO₂ 30–35 mmHg) causes a marked (40–50%) reduction in Kv currents amplitude, and chronically hypoxic animals have a significantly more positive (–43.5 ± 2 mV) resting membrane potential in PASMCs (Smirnov et al., 1994).

As previously noted, substantial evidence suggests that Kv channel inhibition involves ROS. The precise role of ROS, however, remains controversial. Such a response may be mediated by a decrease in H₂O₂ due to a reduction in superoxide (SO⁻) production by the mitochondrial electron transport chain (mETC) due to the decreased O₂ supply (Weir and Archer, 1995; Archer and Michelakis, 2002). Others have alternatively shown that ROS may increase during hypoxia (Waypa et al., 2006). Inhibition of Kv currents by mETC inhibitors, particularly those acting at complexes I and III, supports this concept; at the same time, mitochondrial uncouplers, which decrease mitochondrial membrane potential (ΔΨₘ) (Yuan et al., 1996), and metabolic inhibition of PASMCs with 2-deoxyglucose (Yuan et al., 1994) also inhibit Kv currents, suggesting the presence of multiple, presently undetermined, mitochondria-dependent mechanisms that affect Kv currents in PASMCs. Data supporting the mitochondria as an O₂ sensor in PASMCs were recently published and demonstrated a mitochondrial-dependent regulation of Kv channels in PASMC, a mechanism that was absent in MASMCs and involves increased cellular Mg²⁺ (Firth et al., 2008; Firth et al., 2009). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase presents as another potential oxygen sensing mechanism, as its presence increases SO⁻ production in response to hypoxia (Marshall et al., 1996). When wild type and gp91phox knock out (KO) mice were exposed to hypoxia, however, HPV still occurred in whole lung preparations despite an attenuation of SO⁻ production (Archer et al., 1999).

In addition, Kv channels themselves have been postulated to sense and react to changes in O₂ tension. Several attempts to identify the molecular components of native O₂-sensitive Kv channel currents have been performed (Patel et al., 1997). All studies verify a potential role for the Kv α subunits Kv1.2, Kv1.5, Kv2.1, Kv3.1b, and Kv9.3, which display similar hypoxic inhibition and are all slowly inactivating voltage-gated channels that are sensitive to 4-AP, but not ChTX. Several β subunits are expressed in PASMCs; they potentially interact with the Kv1 family as oxidoreductase enzymes and can function as redox sensors (Osipenko et al., 2000; Coppock and Tamkun, 2001). β subunits may have a significant role in O₂ responses, as the Kv1.2β subunit demonstrates O₂ sensitivity on the Kv4.2α subunit (Archer et al., 2004). Co-expression of any of the β subunits with one of the O₂ sensitive α subunits affects the time- and voltage-dependant properties of the resulting current, suggesting that the α subunits may not be the only O₂ sensors. These results further implicate a role for the β subunit in O₂ sensing in PAs (Coppock and Tamkun, 2001). Using genetically modified mice lacking the O₂ sensitive Kv channel, Kv1.5, Archer and colleagues were able to show an attenuation of HPV (Archer et al., 2001).

**Pulmonary arterial hypertension (PAH)**

The progressive nature of PAH means that it often leads to death due to right ventricular failure. Proliferative apoptosis-resistant cells prevail in pulmonary vascular disease and
significantly contribute to the remodeling of the pulmonary vasculature eventually causing sustained elevation of pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR). In PAH progressive pulmonary vascular remodeling is primarily due to hypertrophy of the medial layer of the pulmonary artery; other related pathological changes include intimal fibrosis, adventitial proliferation, and plexiform lesions. An imbalance in the proliferative and apoptotic rates thus jeopardizes the structural integrity of the pulmonary arteries. Inhibition of K⁺ efflux pathways presents a pharmacological target for pulmonary vascular disease. Induction of PASMC apoptosis has been shown to regress remodeled pulmonary arteries (Cowan et al., 2000), and a fine balance between cell proliferation and apoptosis is critical to maintain normal tissue homeostasis.

1. Increased PASMC proliferation and decreased apoptosis in PAH

Intracellular ion homeostasis, determined by the activity of plasmalemmal ion channels and transporters, is the predominant mechanism regulating apoptotic volume decrease (AVD), which is the early phase of apoptosis. During homeostasis cytoplasmic K⁺ (140 mM) and Cl⁻ (~50–100 mM) concentrations are high, thus creating an electrochemical gradient driving K⁺ efflux and initiating AVD (Kim et al., 1991; Kluck et al., 1997). An osmolarity imbalance created by the extrusion of K⁺ and Cl⁻ causes water to leave the cell through aquaporins, thus causing cell shrinkage (Bortner et al., 1997; Krick et al., 2001a, 2001b). K⁺ efflux has been shown to occur via sarcolemmal K⁺ channels; studies in lymphocytes, neurons, and vascular smooth muscle cells support an early increase in K⁺ conductivity via TEA-sensitive Kv channels in apoptosis (Bortner et al., 1997; Yu et al., 1999; Krick et al., 2001a, 2001b; Platoshyn et al., 2002).

This early stage of apoptosis must occur for progression to the later phases, including DNA fragmentation, caspase activation and mitochondrial membrane depolarization. Decreased intracellular K⁺ concentration relieves the inhibition of endogenous caspases and nucleases allowing nuclease-induced chromatin fragmentation to occur. Resting intracellular K⁺ concentrations are sufficient to inhibit endogenous nucleases and chromatin fragmentation induced by nucleases (Montague et al., 1999). Furthermore, increased intracellular K⁺ concentration reduces the transmembrane potential for K⁺ efflux, thus inhibiting apoptosis through the death receptor-mediated pathway before cytochrome c release and caspase-8 activation (Cain et al., 2001). Increased extracellular K⁺ concentrations also prevent CD95-induced release of cytochrome c (Thompson et al., 2001). A second phase of K⁺ efflux via plasmalemmal K⁺ channels is required after cytochrome c release since an increased Kv current occurs in response to cytochrome c (Krick et al., 2001a; Platoshyn et al., 2002). Studies have shown that overexpression of the Kv1.5 channel-encoding gene, KCNA5, enhance apoptosis and increases caspase-3 activity in PASMC. Inhibition of Kv channels by 4-AP prior to induction of apoptosis by ST results in a reduction of AVD and inhibition of apoptosis by 46% (Ekhterae et al., 2001; Brevnova et al., 2004). In PAH, closure of Kv channels therefore increases intracellular K⁺ inhibiting caspases and suppressing cellular apoptosis (Yu et al., 1997; Remillard and Yuan, 2004).

2. Decreased expression and function of Kv channels in PAH

Inhibition of Kv channel expression and function in PASMCs has been observed in patients...
with idiopathic PAH (IPAH). Kv 1.5 mRNA levels are substantially lower in PASMCs from patients diagnosed with IPAH as compared to normotensive patients and those with secondary pulmonary hypertension (SPH) (Yuan et al., 1998a). Furthermore, the whole cell Kv current is dependent upon plasmalemmal surface expression of functional Kv channels. In IPAH, the amplitude of steady-state Kv currents is significantly lower than that in PASMCs from patients with SPH-PASMCs. The attenuated Kv current is likely to have an etiological role in the development of IPAH. In a subsequent study it was observed that the Kv1.5 encoding gene (KCNA5) of IPAH patients possesses several novel single nucleotide polymorphisms (SNPs) that may correlate with the altered expression and/or function of Kv1.5 channels in PASMCs from these patients (Remillard et al., 2007). Increased risk of PAH is also associated with appetite suppressants such as fenfluramine. Fenfluramine has been shown to increase PAP, and in PASMCs, 60–72 hours exposure lowered mRNA and protein concentrations of Kv1.5 by ~50%. This decreased Kv1.5 expression is likely to cause membrane depolarization and elevated cytosolic Ca\(^{2+}\) concentrations (Wang et al., 1998).

Persistent pulmonary hypertension of the newborn (PPHN)

Like PAH, PPHN is associated with significant short- and long-term morbidity. PPHN occurs when the pulmonary circulation fails to respond to natural stimuli, such as increased oxygen tension, ventilation and shear stress. An adaptive shift from the high resistance state in utero to a postnatal low resistance system fails to occur, thus preventing essential pulmonary gas exchange and oxygenation. Inhalations of oxygen or nitric oxide are typical strategies for improving the extracorporeal membrane oxygen. More recently, reduction of ROS using superoxide dismutase has been shown to improve eNOS activity and expression and ultimately lead to desirable pulmonary vasodilatation (Lakshminrusimha et al., 2006; Farrow et al., 2008).

Recently, the impairment of Kv channel function by oxidative stress was assessed using a fetal lamb model of PPHN (Konduri et al., 2009). This study showed that PA ring contraction due to Kv channel inhibition by 4-AP was significantly attenuated in PPHN. The response, however, was improved by SO\(^{-}\) scavenging. Additionally, 4-AP sensitive Kv currents in PASMC were attenuated in PPHN and restored by scavenging SO\(^{-}\) (Konduri et al., 2009). This study concluded that using SO\(^{-}\) scavengers in the treatment of PPHN might be due in part to the restoration of Kv channel function.

Clinical implications for the Kv channel

Given their central role in the maintenance of cellular homeostasis, ion channels and consequential signaling cascades are potential sites for therapeutic intervention in the treatment of pulmonary vascular disease.

Gene therapy
1. Survivin

McMurtry and colleagues discovered that survivin, an “inhibitor of apoptosis” protein, was only expressed in the resistance arteries from PAH patients who had undergone vascular
remodeling (McMurtry et al., 2005). The same phenomenon was observed in rats with monocrotaline-induced PAH. The study utilized an inhaled adenoviral gene therapy using a mutant form of survivin (survivin-M). They achieved a therapeutic benefit, increasing survival by 25%, via the induction of mitochondrial-dependent apoptosis in association with the activation of Kv channels. In vivo and in vitro effects included lower PVR, RV hypertrophy, and PA medial hypertrophy, induction of PASMC apoptosis, decreased PASMC proliferation, depolarization of the mitochondria causing release of cytochrome c, and increased Kv channel currents (McMurtry et al., 2005). The lack of survivin expression in healthy tissues makes it very attractive as a potential therapeutic approach for PAH.

2. Kv1.5

Pozeg and colleagues provided the first example of a K+ channel gene therapy for a vascular disease in 2003. They utilized a nebulized adenoviral gene therapy approach using Kv1.5 and demonstrated restoration of the O2-sensitive K+ current of PASMCs (Pozeg et al., 2003). While highlighting the feasibility of Kv1.5 gene transfer via airway nebulization in vivo, the study also demonstrated limitations in that using adenovirus resulted in limited-duration transgene expression. Future studies may focus on cell-specific promoters for specific targeting of the gene or integrating vectors such as lentivirus for more stable expression of the transgene.

Activation of Kv7 channels

Kv7 channels (also known as KCNQ channels) have recently been proposed to play a role in the development and progression of PAH. Flupirtine is a Kv7 channel activator that has been recently shown to attenuate the development of CH-induced PAH and to reverse PAH in mouse models of the disease (Morecroft et al., 2009). This interesting study demonstrated for the first time that Kv7 channel agonists may be therapeutically beneficial in PAH.

Dichloroacetate (DCA)

Dichloroacetate (DCA) is a small molecule inhibitor of mitochondrial pyruvate dehydrogenase kinase (PDK). Phosphorylation by PDK inhibits pyruvate dehydrogenase (PDH). PDH is the gatekeeper for fueling mitochondrial oxidative phosphorylation. When PDH is inhibited, pyruvate remains in the cytoplasm and lactate dehydrogenase instigates the production of lactate from pyruvate. Interestingly, the beneficial effects of DCA in PH were independent of such metabolic mechanisms. DCA was effective in three different animal models of PH (hypoxia-induced, monocrotaline-induced, and the fawn-hooded rat) (Bonnet et al., 2006). Long-term dosing of DCA both prevented and reversed chronic hypoxia-induced pulmonary hypertension by restoring K+ currents and Kv2.1 channel expression in PASMCs (Michelakis et al., 2002). A subsequent study confirmed the therapeutic benefit of DCA by showing a reversal of the Kv1.5 channel downregulation in resistance PAs. PA remodeling was attenuated due to an increase in the mitochondria-dependent ratio of apoptosis to proliferation and an increase in functional Kv1.5 channels (McMurtry et al., 2004). One therapeutic advantage for DCA is that it seems to uniquely target the dysfunctional mitochondria with no apparent effects on normal cells (Bonnet et al., 2007).
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