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REVIEW

Potassium channels in the regulation of pulmonary artery smooth muscle cell proliferation and apoptosis: pharmacotherapeutic implications

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Maintaining the proper balance between cell apoptosis and proliferation is required for normal tissue homeostasis; when this balance is disrupted, disease such as pulmonary arterial hypertension (PAH) can result. Activity of K^+ channels plays a major role in regulating the pulmonary artery smooth muscle cell (PASMC) population in the pulmonary vasculature, as they are involved in cell apoptosis, survival and proliferation. PASMCs from PAH patients demonstrate many cellular abnormalities linked to K^+ channels, including decreased K^+ current, downregulated expression of various K^+ channels, and inhibited apoptosis. K^+ is the major intracellular cation, and the K^+ current is a major determinant of cell volume. Apoptotic volume decrease (AVD), an early hallmark and prerequisite of programmed cell death, is characterized by K^+ and Cl^- efflux. In addition to its role in AVD, cytosolic K^+ can be inhibitory toward endogenous caspases and nucleases and can suppress mitochondrial cytochrome *c* release. In PASMC, K^+ channel activation accelerates AVD and enhances apoptosis, while K^+ channel inhibition decelerates AVD and inhibits apoptosis. Finally, inhibition of K^+ channels, by increasing cytosolic [Ca^{2+}] as a result of membrane depolarization-mediated opening of voltage-dependent Ca^{2+} channels, leads to PASMC contraction and proliferation. The goals of this review are twofold: (1) to elucidate the role of K^+ ions and K^+ channels in the proliferation and apoptosis of PASMC, with an emphasis on abnormal cell growth in human and animal models of PAH, and (2) to elaborate upon the targeting of K^+ flux pathways for pharmacological treatment of pulmonary vascular disease.
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Abbreviations: 4-AP, 4-aminopyridine; BMP, bone morphogenetic protein; DCA, dichloroacetate; MCT, monocrotaline; PAH, pulmonary arterial hypertension; PASMC, pulmonary artery smooth muscle cell; ST, staurosporine; VDCC, voltage-dependent Ca^{2+} channels

Introduction

A proper balance between cell death and proliferation is necessary for the normal development and function of tissues. Disturbing this equilibrium can lead to disease states, such as cancer (Green and Evan, 2002), neurodegenerative disorders (Yuan and Yankner, 2000) and pulmonary hypertension (Mandegar *et al.*, 2004). Studies have begun to elucidate the roles of ions, including K^+, Ca^{2+}, Na^+ and Cl^-, and their channels, in both apoptosis and cell proliferation, although much remains unknown. K^+ channels are found on both the plasma membranes and internal membranes of many cell types where they respond to a variety of stimuli. The four main functional classes are (*i*) voltage-gated (K_V) K^+ channels; (*ii*) Ca^{2+}-activated (K_{Ca}) K^+ channels, which are further divided into small-conductance (SK), intermediate-conductance (IK) and large-conductance (BK or maxiK) subfamilies; (*iii*) inwardly rectifying (K_{IR}) K^+ channels, of which ATP-sensitive (K_{ATP}) K^+ channels are a member, composed of $K_{IR}6.x$ and sulphonylurea subunits; and (*iv*) two-pore domain (K_{2P}) K^+ channels. Diversity of K^+ channels is due to a number of factors, including a multitude of encoding genes (over 75 have been identified so far), the heterotetrameric structure of functional channels, and the fact that channels can associate with both accessory β regulatory subunits and electrically silent membrane subunits that alter channels' physiological properties (Coetzee *et al.*, 1999; Amberg *et al.*, 2003).

Traditionally, K^+ channels are associated with repolarization after action potentials and setting the resting membrane potential of excitable cells (Nelson and Quayle, 1995; Yuan, 1995; Yuan *et al.*, 1998c; Hille, 2001; Amberg *et al.*, 2003), but they are also implicated in T-lymphocyte activation (Chandy

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et al., 1984; DeCoursey *et al*., 1984), hypoxic pulmonary vasoconstriction (Yuan *et al*., 1993; Smirnov *et al*., 1994; Archer *et al*., 1998; Coppock *et al*., 2001; Yuan, 2001), myocardial protection against ischaemia (Nichols and Lederer, 1991; Yokoshiki *et al*., 1998), controlling insulin release from pancreatic β cells (Yokoshiki *et al*., 1998; Thevenod, 2002) and neurotransmitter release (Rudy, 1988; Toro *et al*., 1998). Findings within the last two decades have also implicated K^+ channels and K^+ flux in cell volume regulatory mechanisms, as well as both programmed cell death (apoptosis) and cellular proliferation. Inhibition of channels is associated with proliferation of pulmonary artery smooth muscle cells (PASMCs) (Platoshyn *et al*., 2000), whereas in certain other cell types, proliferation has been found to be linked with K^+ efflux (Wonderlin and Strobl, 1996; Pardo, 2004). Activation of K^+ channels, leading to K^+ efflux, is associated with apoptosis through two main mechanisms. Although activity of the electrogenic Na^+/K^+ pump plays a major role in regulating the transmembrane K^+ gradient, apoptotic volume decrease (AVD), a necessary prerequisite for cells undergoing apoptosis, is caused mainly by K^+ loss (Bortner *et al*., 1997; Maeno *et al*., 2000). Additionally, a loss of K^+ releases inhibition of cytoplasmic caspases (Hughes *et al*., 1997; Dallaporta *et al*., 1998). A proper balance between proliferation and apoptosis is required for normal tissue homeostasis; when this balance is disturbed in favour of proliferation or too little apoptosis, the resultant increase in cell population can cause pathophysiological problems, as in pulmonary arterial hypertension (PAH).

The pulmonary circulation is a high-flow, low-resistance and low-pressure system, the vascular resistance of which is determined by the contractility of the PASMCs of the medial layer. Sustained pulmonary vasoconstriction can cause increased pulmonary vascular resistance by narrowing the lumen of pulmonary arteries. Another of the major pathologies of PAH is pulmonary vascular medial thickening, caused by PASMC hypertrophy and hyperplasia. Abnormal apoptotic or cell proliferation regulation has been observed in PASMC from PAH patients (Rubin, 1997; Mandegar *et al*., 2004). This review focuses on K^+ channels in PASMC proliferation and apoptosis, with attention given to the dysregulation of K^+ equilibrium in PAH and the possibility of therapeutic targeting of K^+ channels to treat PAH.

Role of K^+ channels in PASMC

Intracellularly, K^+ is the major cationic species because of the activity of the Na^+/K^+ ATPase. At rest, the plasma membrane is most permeable to K^+; therefore, the activity of K^+ channels plays a large role in setting the resting membrane potential (E_m) (Nelson and Quayle, 1995; Yuan, 1995; Peng *et al*., 1996; Archer *et al*., 1998; Yuan *et al*., 1998c; Amberg *et al*., 2003). Although K_V channels have been studied most extensively in this context (Nelson and Quayle, 1995; Yuan, 1995; Peng *et al*., 1996; Archer *et al*., 1998; Yuan *et al*., 1998c), K_{Ca}, K_{ATP} and K_{2P} channels have also been implicated in setting E_m in PASMC (Smirnov *et al*., 1994; Nelson and Quayle, 1995; Peng *et al*., 1996; Yokoshiki *et al*., 1998; Gurney *et al*., 2003; Olschewski *et al*., 2006). Partly due to the electrogenic nature of the Na^+/K^+ ATPase, pumping three Na^+ out of the cell for every two K^+ it brings into the cell, the membrane potential is negative at rest. The following equation describes whole-cell K_V currents: $I_{K(V)} = N \times i \times P_{open}$, where '$N$' is the total number of functional K_V channels in the plasma membrane, 'i' is the amplitude of single-channel current and 'P_{open}' is the steady-state open probability of the channel. The electrochemical gradient of K^+ is directed out of the cell, so when K^+ channels open, K^+ flows out, leading to a membrane hyperpolarization. Conversely, a decrease in the outward current through K^+ channels, due to decreased open probability, single-channel current and/or number of available channels, will lead to membrane depolarization.

Voltage-gated K^+ channels play an important role in regulating pulmonary vascular tone through the control of E_m, an important determinant of cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$) in PASMC (Fleischmann *et al*., 1994; Smirnov *et al*., 1994; Nelson and Quayle, 1995; Yuan, 1995; Carl *et al*., 1996; Archer *et al*., 1998; Yuan *et al*., 1998c), although other K^+ channels are also known to play a role in controlling vascular tone in other cell types (Yokoshiki *et al*., 1998). When $I_{K(V)}$ is inhibited pharmacologically, for example, by 4-aminopyridine (4-AP), the resulting membrane depolarization opens L-type voltage-dependent Ca^{2+} channels (VDCC), causing Ca^{2+} influx and an increase in $[Ca^{2+}]_{cyt}$ (Nelson *et al*., 1990; Yuan, 1995; Yuan *et al*., 1996; Platoshyn *et al*., 2000; Cribbs, 2006), and subsequent smooth muscle cell contraction through Ca^{2+}–calmodulin activation of myosin light-chain kinase (Somlyo and Somlyo, 1994; Ratz *et al*., 2005). Conversely, activation of K^+ channels in PASMC, such as that induced by nitric oxide (NO), hyperpolarizes the membrane and decreases $[Ca^{2+}]_{cyt}$ (Nelson *et al*., 1990; Yuan *et al*., 1996). Furthermore, it has been shown that blockade of K_V channels with 4-AP in isolated pulmonary arterial rings is sufficient to increase arterial tension and to inhibit NO-induced relaxation (Peng *et al*., 1996; Yuan *et al*., 1996; Zhao *et al*., 1997). These data support the hypothesis that K_V channels are important contributors to E_m in PASMC, which in turn, is a major determinant of $[Ca^{2+}]_{cyt}$ (Figure 1).

In addition to K_V channels, K_{Ca} and K_{IR} channels have also been shown to contribute to regulating E_m of arterial smooth muscle cells (Nelson and Quayle, 1995). Additionally, the expression of different K^+ channel subtypes may also influence cell phenotypes. In fully differentiated aortic smooth muscle cells (that is, those having the contractile phenotype), large-conductance BK (BK_{Ca}) currents predominate, whereas in immature cells (that is, of the proliferative phenotype), voltage-insensitive intermediate-conductance IK_{Ca} currents are the dominant type (Neylon *et al*., 1999; Köhler *et al*., 2003; Jackson, 2005). Ivanov *et al*. (2006) found that epidermal growth factor (EGF) caused membrane hyperpolarization of freshly isolated arterial smooth muscle cells through iberiotoxin-sensitive BK_{Ca} channels. Similarly, K_V channels are also found to be differentially expressed according to the cell's phenotype. K_V1 channels were found to be associated with the contractile phenotype of human uterine vascular smooth muscle cells, whereas $K_V3.4$ was associated with the proliferating phenotype (Miguel-Velado *et al*., 2005).

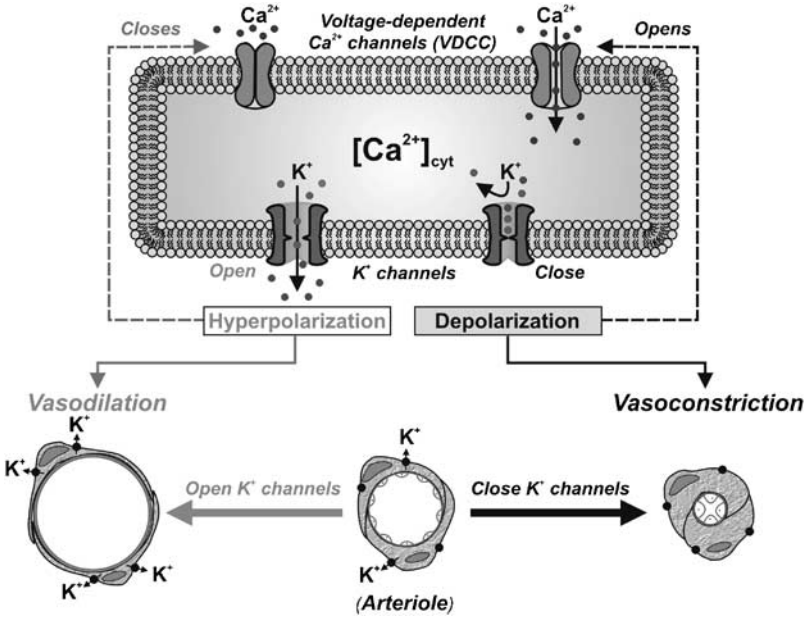

Figure 1 Proposed mechanisms showing the role of K^+ channel activity and cytosolic $[Ca^{2+}]$ in vasoconstriction. When K^+ channels are blocked (or K^+ channel expression is downregulated), the resulting membrane depolarization opens voltage-dependent Ca^{2+} channels (VDCC), promotes Ca^{2+} influx, increases $[Ca^{2+}]_{cyt}$ and causes vasoconstriction. When K^+ channels are activated (or K^+ channel gene expression is upregulated), the membrane hyperpolarization closes VDCC, inhibits agonist-mediated Ca^{2+} influx and causes vasodilation.

When K_V channels close and $[Ca^{2+}]_{cyt}$ increases, smooth muscle contraction is not the only functional change that occurs. Intracellular Ca^{2+}, in addition to its role in muscle contraction, is also an important second messenger for cell migration and proliferation. Ca^{2+} influx through L-type VDCC triggers CRE- and c-Jun-mediated gene transcription, and activates transcription factors, such as CREB, NF-AT and NF-κB that are involved in cell proliferation, protein synthesis and inflammation (Sheng *et al*., 1990; Bading *et al*., 1997; Hardingham *et al*., 1997, 1998; Hardingham and Bading, 1999). Ca^{2+} is also required for cell-cycle progression, for example, at the G_0 to G_1 transition, DNA synthesis and mitosis (Dubois and Rouzaire-Dubois, 1993; Berridge, 1995; Clapham, 1995). In PASMC, inhibition of K_V channels is associated with cell proliferation, consistent with the idea that the depolarization-induced increase in $[Ca^{2+}]_{cyt}$ allows for progression through the cell cycle. Specifically, Platoshyn *et al*. (2000) showed that in proliferating PASMC compared to growth-arrested PASMC, resting $[Ca^{2+}]_{cyt}$ was higher and the E_m more depolarized. Furthermore, $I_{K(V)}$ was diminished in proliferating PASMC, consistent with the hypothesis that Ca^{2+} influx following inhibition of K^+ currents leads to proliferation. Thus, a rise in $[Ca^{2+}]_{cyt}$ also functions as a signal for stimulating PASMC proliferation and gene expression (Figure 2).

It should be noted that evidence as to which K^+ channels are involved in specific physiological roles, such as setting the E_m or cell volume control, can be extremely cell specific, even within PASMC, as cultured proliferating cells may express a K^+ channel profile that is distinct from that expressed by freshly isolated PASMC (Neylon *et al*., 1999; Moudgil *et al*., 2006). Furthermore, different segments (for example, conduit vs resistance arteries) of the vessels within

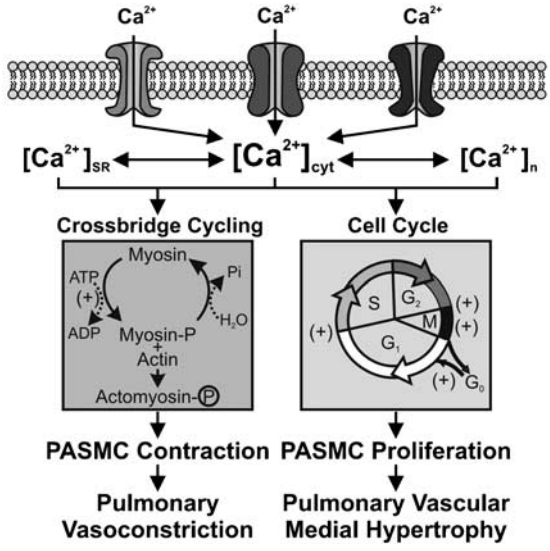

Figure 2 Ca^{2+} causes pulmonary artery smooth muscle cell (PASMC) contraction and stimulates cell proliferation. An increase in $[Ca^{2+}]_{cyt}$ due to Ca^{2+} influx through voltage-dependent Ca^{2+} channels (such as L-type and T-type VDCC) causes PASMC contraction by promoting myosin and actin interaction and stimulates PASMC proliferation by propelling the cell through the cell cycle. (+) Ca^{2+}-sensitive steps in the cell cycle. In addition, increases in $[Ca^{2+}]$ in the sarcoplasmic reticulum (SR, $[Ca^{2+}]_{SR}$), cytosol ($[Ca^{2+}]_{cyt}$) and nucleus ($[Ca^{2+}]_n$), all contribute to activating cytoplasmic signalling proteins and nuclear transcription factors (for example, CREB, NF-AT, c-Jun and NF-κB) and stimulating cell proliferation.

the pulmonary circulation, and even different PASMC from the same segment (for example, in different cell-cycle phases or in different phenotypes), express varying profiles of K^+

channels (Archer *et al*., 1996). Additionally, effects of ion channels in response to apoptotic or proliferative stimuli are likely to be highly time dependent, as initial short-term effects develop within minutes, whereas long-term effects can take hours or days to occur.

Cell volume is directly related to the movement of ions, with homeostasis being achieved by a balance of osmotic pressure across the plasma membrane. Most cells achieve and maintain their osmotic balance due to the continuous activity of the Na^+/K^+ ATPase pump, which creates an intracellular environment rich in K^+ and low in Na^+, which is in contrast with the extracellular space where high levels of Na^+ and low levels of K^+ exist. Despite the net transmembrane potential that is generated, a net electrochemical gradient is established that favours the passive movement of K^+ out of the cell (al-Habori, 1994). Because most cell types have a high resting permeability to K^+, resting E_m values are generally close to those of the actual Nernstian K^+ potential ($E_K \approx -80$ mV). However, the distinct permeability of the membrane to other ions (for example, Ca^{2+}, Na^+ and Cl^-) in different cell types, such as smooth muscle cells, can render E_m more positive than E_K. Nonetheless, it is not surprising that K^+ channels are also involved in the regulation of cell volume (Figure 3a). Cells have active volume regulatory mechanisms, including regulatory volume decrease (RVD), to maintain a constant volume in response to environmental challenges such as hypo- or hypertonic challenge and the gain or loss of osmotically active substances by transport or metabolism (Chamberlin and Strange, 1989). Interestingly, lymphocytes lack a volume regulatory mechanism and undergo apoptosis in response to hypertonic conditions, whereas other cell types that initially undergo shrinkage upon hypertonic challenge are able to then maintain their volume or regulate back to normal volumes without undergoing apoptosis (Bortner and Cidlowski, 1996). Imbalances in osmolarity across cell membranes trigger the movement of water through aquaporins, with the resulting hydrostatic pressure gradient compensated by the movement of ions, namely K^+ and Cl^- (Figure 3a) (Lang *et al*., 1998).

Various K^+ channels are involved in regulation of cell volume, including K_V channels (for example, $K_V1.3$ and $K_V1.5$), K_{Ca} channels (for example, SK_{Ca}, IK_{Ca} and BK_{Ca}), and K_{2P} (for example, TREK1, TRAAK and TASK2) (Felipe *et al*., 1993; Lang *et al*., 1998). An increased IK_{Ca} current was observed during the RVD response to hypotonic challenge in human epithelial small intestine cells; treatment with clotrimazole, an IK_{Ca} channel-specific blocker, both inhibited the currents and prevented the RVD response (Wang *et al*., 2003). Additionally, BK_{Ca} currents in human bronchial epithelial cells were augmented in response to hypotonicity (Fernández-Fernández *et al*., 2002). As K^+ flows out of the cell in response to hypotonicity, water follows and the osmotic balance between the intra- and extracellular environments is restored. In addition to maintaining cell volume, K^+ channels and volume regulation also play a role in controlling the progression of apoptosis (Remillard and Yuan, 2004; Burg *et al*., 2006). The role of K^+ in volume regulation in relation to apoptosis will be discussed in further detail below.

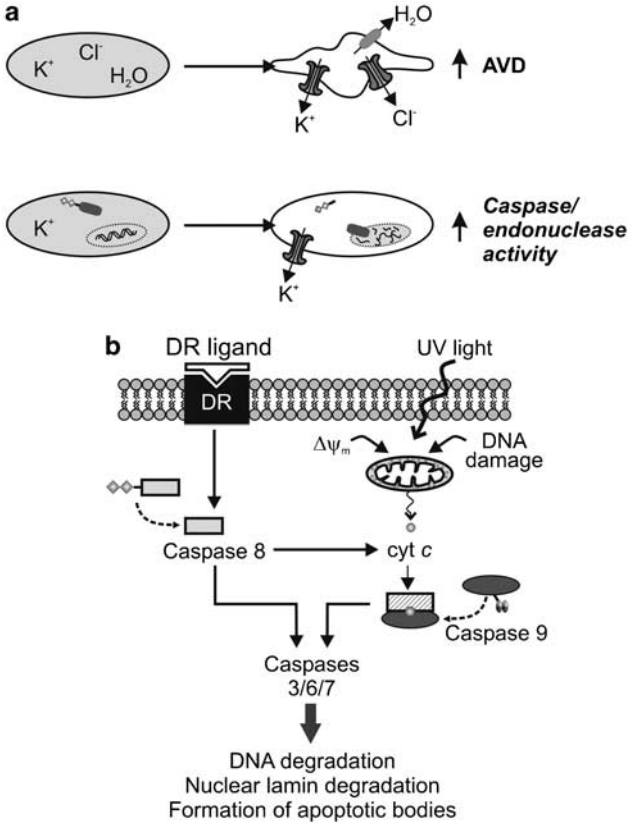

Figure 3 Role of K^+ efflux in regulation of apoptotic volume decrease (AVD) and apoptosis. (**a**) Opening of K^+ channels in the plasma membrane leads to K^+ (and Cl^-) efflux. The resultant gradient of osmolarity causes outward transportation of water and eventually leads to AVD (upper panel). Decreased cytosolic [K^+] due to increased K^+ efflux would relieve the inhibitory effect of cytoplasmic K^+ on cytoplasmic cascases and endonucleases, and promote apoptosis (lower panel). (**b**) Two pathways of apoptosis. When death receptors (DRs) are activated (for example, by Fas ligand), cleavage of procaspase 8 (and/or 10) to active caspase 8 is an important initial step to induce apoptosis. Cytochrome *c*, which can be released from the mitochondria to the cytosol when cells are exposed to UV light or when mitochondrial membrane potential ($\Delta\Psi_m$) is depolarized, activates cytoplasmic caspase 9. Active caspases 8 and 9 then activate caspases 3/6/7 and cause DNA fragmentation and nuclear breakage, and eventually cell death.

Overview of apoptosis

Apoptosis, or programmed cell death, is an important part of normal tissue development and function that allows cells to die by a regulated series of events. Morphological changes observed in apoptotic cells include loss of cell volume, termed AVD, nuclear condensation, DNA fragmentation and apoptotic body formation. Apoptosis can be divided into three general stages: (1) the initiation event, when the signal to apoptose is first received by the cell and AVD begins; (2) the effector phase when the mitochondrial membrane potential ($\Delta\Psi_m$) is depolarized, cytochrome *c* is released and caspases are activated; and (3) the last phase in which DNA is degraded, apoptotic bodies pinch off from the plasma membrane, the nuclear lamina is degraded, the cytoskeleton is broken down and phosphatidyl serine, normally found only on the internal face of the plasma membrane, is

exposed to the external environment. The two major apoptotic pathways, the mitochondrial (intrinsic) and death receptor (extrinsic) pathways, culminate in the activation of cytoplasmic effector caspases (caspases 3, 6 and 7) from their inactive procaspase forms (Haunstetter and Izumo, 1998). Once activated, effector caspases cleave chromatin, proteins and the nuclear lamin (Thornberry and Lazebnik, 1998). Although the effector caspases activated by the two pathways are identical, the initiator caspases, which activate effector caspases, are distinct: the extrinsic pathway involves initiator caspase 8 (and 10), while the intrinsic pathway involves caspase 9 (Vu *et al*., 2001; Gustafsson and Gottlieb, 2007) (Figure 3b).

The extrinsic pathway starts with the activation of transmembrane death receptors by ligands such as Fas, TNF-α or CD95 that ultimately leads to the activation of effector caspases through the recruitment and activation of death domain proteins and initiator caspase 8 (Gulbins *et al*., 2000; Vu *et al*., 2001; Remillard and Yuan, 2004; Gustafsson and Gottlieb, 2007). This pathway can also cause the release of cytochrome *c* from the mitochondria.

The intrinsic pathway involves the release of cytochrome *c* from the mitochondrial matrix and can be induced by such mediators as staurosporine (ST), UV radiation and actinomycin D (Green and Reed, 1998; Duchen, 1999). Release of cytochrome *c* is linked to $\Delta\Psi_m$ disruption and the formation of the mitochondrial permeability transition (MPT) pore (Kroemer *et al*., 1998). Formation of the MPT and $\Delta\Psi_m$ disruption mark a point of no return after which the cell is committed to apoptose (Kroemer *et al*., 1998; Duchen, 1999). Opening of the MPT results in cytochrome *c* release, uncoupling of the respiratory chain, reduction of $\Delta\Psi_m$ and swelling of the mitochondrial matrix (Gulbins *et al*., 2000). Once released into the cytosol, cytochrome *c* associates with APAF-1 and initiator caspase 9 to form the apoptosome, which activates effector caspases that lead to chromatin degradation and apoptosis. The role of intracellular K^+ in inhibiting caspases will be discussed in the next section.

The MPT can also associate with members of the Bcl-2 family of proteins (Gulbins *et al*., 2000). Proapoptotic Bax and Bak form a pore through which, upon $\Delta\Psi_m$ depolarization, cytochrome *c* can pass from the matrix to the cytosol (Shimizu *et al*., 2000). Anti-apoptotic proteins such as Bcl-2 and Bcl-x_L have their effects by promoting mitochondrial hyperpolarization and preventing the opening of the MPT, which attenuates cytochrome *c* release (Vander Heiden *et al*., 1999; Gustafsson and Gottlieb, 2007). Overexpression of Bcl-2 can inhibit mitochondrial membrane depolarization and cytochrome *c* release in response to *tert*-butylhydroperoxide challenge, but does not protect against diamide-induced $\Delta\Psi_m$ depolarization or cytochrome *c* release (Zamzami *et al*., 1998), indicating that different apoptotic inducers function with distinct mechanisms. Bcl-2 can also suppress the activity of proteases activated by apoptotic signals. In epithelial cells, which normally undergo apoptosis in response to disruption of cell adhesions in a process called anoikis, Bcl-2 overexpression was found to suppress activity of an interleukin-converting enzyme-related cysteine protease in response to cell suspension (Frisch *et al*., 1996). In PASMC, the anti-apoptotic effect of Bcl-2 has been linked to a decrease in $I_{K(V)}$ (Ekhterae *et al*., 2001). In cells treated with ST, a potent apoptotic inducer, Bcl-2 both prevented the ST-induced increase in $I_{K(V)}$ and significantly inhibited apoptosis; furthermore, the mRNA expression of pore-forming K_V channel α subunits ($K_V1.1$, $K_V1.5$ and $K_V2.1$) was found to be attenuated when Bcl-2 was overexpressed (Ekhterae *et al*., 2001). Overall, the importance of the mitochondria to apoptosis lies mainly in its release of cytochrome *c*, which activates the final mediators of programmed cell death.

The mitochondrial inner membrane is also host to a variety of K^+ channels that control mitochondrial K^+ uptake, background conductance, volume regulation, and that play a role in protection against ischaemia (Murata *et al*., 2001; Lee and Thevenod, 2006). K^+ channels, including mitoK_{Ca} (Siemen *et al*., 1999; Xu *et al*., 2002) and mitoK_{ATP} (Inoue *et al*., 1991), are similar to their plasmalemmal counterparts in that they are sensitive to increased $[Ca^{2+}]_{cyt}$ and ATP, respectively. Activity of mitoK_{Ca} channels play a role in protection against myocardial infarction (Xu *et al*., 2002). Mitochondrial and plasmalemmal K_{ATP} channels can be distinguished from one another pharmacologically, as mitochondrial channels are selectively stimulated and inhibited by diazoxide and 5-hydroxydecanoate, respectively (Inoue *et al*., 1991; Liu *et al*., 1998; Siemen *et al*., 1999; Deçbska *et al*., 2001; Xu *et al*., 2002).

Apoptotic volume decrease and intracellular K^+ in the early stages of apoptosis

K^+ fluxes have been implicated in both early and late stages of apoptosis. AVD, one of the earliest morphological changes observed in cells undergoing apoptosis, is a requisite for apoptosis (Bortner and Cidlowski, 1996). AVD is accomplished in nearly the same manner as RVD. As K^+ efflux through open K^+ channels increases in the early stages of AVD, Cl^- ions follow, moving down their electrochemical gradient. Water exits the cell through aquaporins to maintain the osmotic pressure balance between the intracellular and extracellular compartments, thus achieving cell shrinkage. Given the similarity of the molecular mechanisms of AVD and RVD, it is not surprising that in certain instances AVD is coupled to facilitated RVD (Maeno *et al*., 2000). AVD can occur within minutes or a few hours of apoptotic induction and occurs before caspase activation, DNA fragmentation and, in some instances, disruption of the mitochondrial membrane potential (Bortner and Cidlowski, 1996; Bortner *et al*., 1997; Yu *et al*., 1997; Bortner and Cidlowski, 1999; Maeno *et al*., 2000; Krick *et al*., 2001a; Platoshyn *et al*., 2002). In some studies, K^+ loss was observed to occur only in cells in which the mitochondrial membrane potential had already been disrupted; however, DNA fragmentation was observed only in cells that had undergone K^+ leakage (Dallaporta *et al*., 1998).

Interestingly, some studies have found that certain apoptotic characteristics (that is, cell shrinkage, K^+ efflux and altered $\Delta\Psi_m$) can occur independently of both DNA degradation and caspase activity (Bortner and Cidlowski, 1999; Platoshyn *et al*., 2002). In PASMC, ST augments $I_{K(V)}$, induces $\Delta\Psi_m$ depolarization and causes apoptosis (Krick

et al., 2001a; Platoshyn *et al*., 2002). However, when cells were exposed to a high concentration of external K^+, ST-induced $\Delta\Psi_m$ still occurred while the ST-induced $I_{K(V)}$ increase and apoptosis were both inhibited (Krick *et al*., 2001a). Furthermore, cytochrome *c*, when introduced into PASMC cytoplasm, was found to increase K^+ currents before inducing nuclear breakage; the rise in K^+ current was independent of caspase 9 activity (Platoshyn *et al*., 2002).

The requirement of K^+ efflux for apoptosis was initially shown in lymphocytes (Bortner *et al*., 1997; Hughes *et al*., 1997), but was later demonstrated in neurons (Yu *et al*., 1997), thymocytes (Dallaporta *et al*., 1998), liver cells (Nietsch *et al*., 2000), cardiomyocytes (Ekhterae *et al*., 2003) and PASMC (Krick *et al*., 2001a, b). In lymphocytes treated with various apoptotic inducers, including Fas ligand, dexamethasone, ST or anisomysin, only the cells that were shrunken exhibited DNA fragmentation, increased caspase activity and depolarized $\Delta\Psi_m$ (Bortner *et al*., 1997; Bortner and Cidlowski, 1999). Furthermore, apoptosis is inhibited when K^+ efflux is prevented, either by raising the extracellular K^+ concentration (that is, decreasing the driving force on K^+ efflux) or by pharmacologically blocking K^+ channels (Bortner *et al*., 1997; Gómez-Angelats *et al*., 2000; Krick *et al*., 2001b). That K^+ efflux, rather than mitochondrial membrane depolarization itself, is necessary for apoptosis in PASMC was demonstrated by Krick *et al*. (2001a), who showed that ST-induced K^+ efflux and apoptosis, but not mitochondrial membrane depolarization, were inhibited by high levels of extracellular K^+. Additionally, PKC has been shown to be inhibitory towards Fas ligand-induced apoptosis through its effects on K^+ loss and cell shrinkage (Gómez-Angelats *et al*., 2000).

K^+ efflux as a requisite for the occurrence of apoptosis may be cell and mediator specific. For example, UV-induced apoptosis of myeloblastic leukaemia cells was found to depend on K^+ channel activation, whereas etoposide induced apoptosis regardless of K^+ channel suppression (Wang *et al*., 1999). Additionally, amyloid β (Aβ) was found to increase outward K^+ currents in certain neuronal cell types; toxicity in a cholinergic cell line was found to depend on activation of tetraethylammonium (TEA)-sensitive K^+ channels, whereas glucose deprivation did not have an effect on K^+ currents, and TEA treatment did not protect the cells from hypoglycaemia death (Colom *et al*., 1998). Furthermore, a dopaminergic cell line was found to be sensitive to Aβ in that the cells died when exposed; however, K^+ current density was not affected by Aβ and TEA did not prevent cell toxicity (Colom *et al*., 1998).

Although increases in K^+ channel activity have been widely associated with apoptotic induction, the converse has also been observed. In Jurkat T lymphocytes, treatment with dexamethasone decreases $I_{K(V)}$ (Lampert *et al*., 2003). These observations do not rule out an early increase in $I_{K(V)}$, however, as cells were pretreated for 2–3 h before observation. Treatment of Jurkat T lymphocytes with ceramide, a metabolite synthesized after Fas receptor activation, decreases $I_{K(V)}$ and phosphorylates and inactivates $K_V1.3$ channels (Szabò *et al*., 1996; Gulbins *et al*., 1997). Apoptosis is a dynamic process, with the final biochemical changes, such as DNA degradation, marking the common manifestations of an apoptotic cell. Given the above data and the complexity of K^+ channels, it is likely that the exact mechanisms leading up to the final common stages of apoptosis are both highly cell and inducing agent specific. Another example that illustrates this point is the effect of overexpression of K_V channels. Interestingly, overexpression of the $K_V1.5$ channel gene (*KCNA5*) in PASMC enhances apoptosis (Brevnova *et al*., 2004), whereas transfection of $K_V10.1$ (*eag*) into CHO cells induces a transformed phenotype (Pardo *et al*., 1999).

The identity of the K^+ currents and channels involved in AVD has been mostly inferred from pharmacological blockade of specific K^+ channels: 4-AP and TEA-sensitive K_V channels (Yu *et al*., 1997; Krick *et al*., 2001a, 2002; Bock *et al*., 2002; Ekhterae *et al*., 2003), quinine- and Ba^{2+}-sensitive K^+ channels (Maeno *et al*., 2000; Nietsch *et al*., 2000) and K_{Ca} channels (Nietsch *et al*., 2000; Krick *et al*., 2001b, 2002; Ekhterae *et al*., 2003). FCCP, a mitochondrial protonophore, was shown to cause apoptosis by increasing K_{Ca} currents ($I_{K(Ca)}$) through BK_{Ca} channels in rat and human PASMC; blockage of these channels with iberiotoxin or TEA inhibited FCCP-induced apoptosis (Krick *et al*., 2001b). Cells lacking $K_V1.3$ are resistant to actinomycin D-induced apoptosis, failing to demonstrate DNA fragmentation, release of cytochrome *c* and depolarization of $\Delta\Psi_m$; retransfection of the missing channel restores the apoptotic response to actinomycin D (Bock *et al*., 2002). In PASMC, K_{Ca} channel opening enhances nitric oxide-mediated apoptosis (Krick *et al*., 2002), and overexpressing $K_V1.5$ enhances both basal and ST-induced apoptoses (Brevnova *et al*., 2004), whereas K^+ channel blocked with 4-AP, TEA or iberiotoxin inhibits ST- and NO-induced apoptoses (Krick *et al*., 2002; Brevnova *et al*., 2004). These studies, taken together, indicate an essential role for increased K^+ flux, most notably through K_V and K_{Ca} channels, in apoptosis.

K^+ in suppression of caspases during mid-to-late phase apoptosis

Intracellular K^+ is inhibitory towards apoptosis in that physiological levels of K^+ inhibit the activation of caspases. Just as blockade of K^+ efflux diminishes AVD, it has also been shown to prevent the activation of cytosolic caspases (Krick *et al*., 2001a). Using dexamethasone-induced thymocyte autodigestion as a model for apoptosis, Hughes *et al*. (1997) demonstrated that only cells with decreased cytosolic K^+ concentration ($[K^+]_{cyt}$) exhibited intracellular caspase and nuclease activity. Maintaining high $[K^+]_{cyt}$ suppressed caspase and nuclease activity independently of the mode of apoptotic induction (Hughes *et al*., 1997). Thompson *et al*. (2001), working with a cell free system, showed that in activated lysates, APAF-1 oligomerized to both an active caspase processing complex and a biologically inactive complex; in the presence of increasing $[K^+]_{cyt}$, the active complex did not form and caspase activation was inhibited. These findings were extended to show that normal intracellular levels of K^+ are enough to suppress active complex formation and that the effects of K^+ on apoptosome assembly are antagonized in a concentration-dependent

manner by cytochrome *c* (Cain *et al.*, 2001). The authors postulated that physiological $[K^+]_{cyt}$ levels safeguard the cell against inadvertent cytochrome *c* release, which would lead to irreversible apoptosome formation (Cain *et al.*, 2001). These data suggest that, in addition to contributing to AVD, K^+ efflux creates a permissible environment for caspase and nuclease activity by relieving inhibition on these apoptotic mediators. Therefore, closure or block of K^+ channels, and consequent reduced K^+ efflux, would attenuate apoptosis by both preventing AVD and by maintaining caspase and nuclease inhibition (see Figure 3a, lower panel).

That ion concentrations and fluxes play a role in mediating apoptosis is widely established. However, the role of ions in apoptosis is highly cell specific. This is clearly illustrated with the effects of ouabain, a Na^+/K^+ ATPase inhibitor, on various cell types. Overall, ouabain has the effect of increasing intracellular Na^+ levels and decreasing $[K^+]_{cyt}$. Treatment of rat aortic vascular smooth muscle cells with ouabain attenuates apoptosis induced by serum withdrawal, ST and okadaic acid due to the resulting high intracellular $[Na^+]/[K^+]$ ratio (Orlov *et al.*, 1999). This effect was found to depend on an early induction of RNA synthesis (Orlov *et al.*, 2000). However, porcine aortic endothelial cells respond to ouabain by undergoing necrotic cell death (Orlov *et al.*, 2004). In cultured cortical neurons, ouabain was found to induce cell death that had features reminiscent of both necrosis and apoptosis, including caspase 3 activation, cytochrome *c* release and DNA laddering. These effects were found to be mediated by intracellular depletion of K^+ and accumulation of Ca^{2+} and Na^+ (Xiao *et al.*, 2002). Ouabain, similarly, induced cell death in canine epithelial kidney cells, but this was found to be independent of the inversion of the intracellular $[Na^+]/[K^+]$ ratio, as was the case with the ouabain-induced necrosis of porcine aortic endothelial cells (Pchejetski *et al.*, 2003; Orlov *et al.*, 2004).

In addition to their role in apoptosis, caspases also contribute to inflammatory signalling. Early studies showed that IL-1β processing and export in macrophages challenged with LPS depends on K^+ efflux (Perregaux and Gabel, 1994; Walev *et al.*, 1995). Bacterial toxin-induced membrane permeabilization of CHO cells leads to decreased $[K^+]_{cyt}$, which promotes both formation of the inflammasome, an innate immune signalling complex, and activation of caspase 1 (Gurcel *et al.*, 2006). Caspase 1 is known to play a role in processing the precursor form of interleukin-1β, a component of inflammation signalling. Interestingly, when activated in the context of bacterial toxin membrane permeabilization and reduced $[K^+]_{cyt}$, caspase 1 promotes membrane repair through the activation of the sterol regulatory element-binding proteins (Gurcel *et al.*, 2006). K^+ efflux was found to be both necessary and sufficient to activate sterol regulatory element-binding protein (Gurcel *et al.*, 2006).

Overall, K^+ fluxes contribute to apoptosis in distinct chronological phases. In the early stages of apoptosis where AVD is most prominent, increased K^+ efflux in response to an apoptotic mediator causes cell shrinkage. Later after K^+ efflux has been initiated during AVD and the osmotic balance is disrupted, a low K^+ level in the cytoplasm creates a permissible environment for caspase activation, thus leading to chromatin and protein cleavage.

K^+ channels in proliferation

The evidence presented until this point is quite strong regarding the role of K^+ channels in vascular smooth muscle cell apoptosis. Conversely, there is also mounting evidence that K^+ channel activation may also play a significant role in promoting proliferation (Neylon, 2002). Traditionally, K^+ channel activation would result in membrane hyperpolarization and decreased Ca^{2+} influx via voltage-gated Ca^{2+} channels. However, it appears that, in some cases, K^+ channel-mediated hyperpolarization can regulate the spatial and temporal organization of Ca^{2+} signalling. Since only certain types of Ca^{2+} signals can lead to the activation of growth-promoting genes (Dolmetsch *et al.*, 1997; Hardingham *et al.*, 1997), the ability of K^+ channels to modulate the amplitude and duration of Ca^{2+} signalling can therefore influence their capacity to alter cell function.

The most common form of intracellular Ca^{2+} signalling involves Ca^{2+} release from the sarcoplasmic reticulum. Global Ca^{2+} transients induced in contractile smooth muscle cells (for example, caffeine causing release from intracellular ryanodine-sensitive Ca^{2+} stores in the form of Ca^{2+} sparks) lead to activation of BK_{Ca} channels and closure of VDCC. In contrast, sustained $[Ca^{2+}]_{cyt}$ (for example, due to IP_3 receptor stimulation) activate IK_{Ca} channels, which are not affected by changes in E_m. The subsequent hyperpolarization increases the driving force for Ca^{2+} influx across its chemical gradient via non-selective cation channels such as receptor-operated Ca^{2+} channels (Golovina, 1999). The resultant sustained or oscillatory increases in $[Ca^{2+}]_{cyt}$ can selectively activate smooth muscle growth mechanisms such as growth factor gene expression, activation of kinases and other processes involved in cell division, and phosphorylation (Dolmetsch *et al.*, 1997; Hardingham *et al.*, 1997; Levitan, 1999).

One of the observations upon which the hypothesis that increased K^+ current is needed for proliferation depends is that blocking K^+ channels inhibits proliferation. It has been suggested that K^+ channel activity is needed specifically for the G_1/S transition (Wonderlin and Strobl, 1996; Pardo, 2004). In addition, 4-AP suppressed human myeloblastic ML-1 cell proliferation by arresting cells in G_1; however, once cells passed the G_1/S boundary, channel blockade had no effect on cell-cycle progression (Xu *et al.*, 1996). Further, blockade of K^+ channels prevents the activation of the ERK-2 mitogenic signalling cascade initiated by epidermal growth factor in ML-1 cells (Xu *et al.*, 1999).

Generalizations arising from these studies need to be made with caution for many reasons. First, tumour cells are not normal with respect to their proliferative phenotype. The role of K^+ channels in tumour cell proliferation and apoptosis is beyond the scope of this review; however, the interested reader is referred to a recent review by Wang (2004). Second, in many studies involving lymphocytes and various cancer cell lines, the concentrations of K^+ channel antagonist needed to inhibit proliferation are higher than the concentrations needed to inhibit the K^+ currents (Wonderlin and Strobl, 1996). Third, studies showing that K^+ channel blockers inhibit proliferation do not consistently demonstrate that the blockers induce depolarization

or have an effect on intracellular Ca^{2+} (Pardo, 2004). For example, in neuroblastoma cells, blockers inhibited proliferation but did not induce membrane depolarization (Dubois and Rouzaire-Dubois, 1993). Furthermore, studies have shown that charybdotoxin, a BK antagonist, blocks progression of T lymphocytes through the G_1 phase; however, this could be due to the fact that charybdotoxin blocked the induction of IL-2 expression, which is needed for G_1 progression, rather than a direct requirement for K^+ efflux (Wonderlin and Strobl, 1996). These observations suggest that channel blockers that inhibit mitogenesis may have their effects through nonspecific mechanisms.

Intracellular K^+ is protective against apoptosis (Hughes *et al*., 1997; Dallaporta *et al*., 1998), an effect that has been discussed in the previous section. It must be noted that an increased K^+ current would, therefore, set up two opposing forces: one towards an increased driving force for Ca^{2+} entry and subsequent proliferation; and one towards volume decrease and a release of inhibition on caspases and nucleases, which would favour apoptosis. At any rate, Ca^{2+} signalling, including spikes, sparks, oscillations and sustained waves, is known to be complex, with both spatial and temporal attributes of the signal contributing to its effect (Berridge, 1995; Carl *et al*., 1996; Jaggar *et al*., 2000); therefore, the mechanisms linking K^+ channels, Ca^{2+} influx and proliferation are likely to be highly cell and signal specific.

K^+ channels, apoptosis and pulmonary arterial hypertension

The pulmonary vasculature is a high-flow, low-resistance system that depends on arterial distensibility and recruitment to adapt to changing loads of increased cardiac output. Vascular SMCs have low rates of proliferation in the vascular wall, but pathological situations cause vascular SMCs to lose their differentiated state and proliferate; overall, this contributes to vascular remodelling observed in diseases such as PAH (Rubin, 1997; Mandegar *et al*., 2004; Cribbs, 2006). Medial hypertrophy and muscularization of the pulmonary arterial wall are among the main pathological findings in patients with PAH.

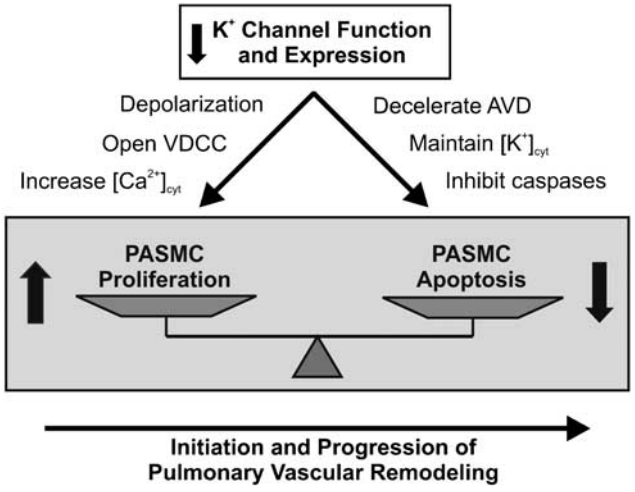

Figure 4 Role of K^+ channels in pulmonary vascular remodelling. Decreased K^+ channel function and expression not only stimulates pulmonary artery smooth muscle cell (PASMC) proliferation by increasing $[Ca^{2+}]_{cyt}$, but also inhibits PASMC apoptosis by decelerating apoptotic volume decrease (AVD) and attenuating cytoplasmic caspase activity. The increased proliferation and inhibited apoptosis in PASMC may play an important role in initiation and/or progression of pulmonary vascular remodelling. VDCC, voltage-dependent Ca^{2+} channels.

Proliferating PASMCs have higher resting $[Ca^{2+}]_{cyt}$ than growth-arrested PASMCs, and an excessive PASMC cell population is one of the hallmarks of PAH. Consistent with these observations, it has been noted that PASMCs from idiopathic PAH (IPAH) patients have a higher resting $[Ca^{2+}]_{cyt}$ and a more depolarized E_m than cells from normal subjects, patients without pulmonary hypertension (NPH) and patients with secondary pulmonary hypertension (SPH) (Yuan *et al*., 1998a, b). Consistent with the role of K_V channels in regulating E_m, PASMCs from IPAH patients also have decreased mRNA levels of $K_V1.5$ and $K_V1.2$, decreased $I_{K(V)}$, and more rapid inactivation of $I_{K(V)}$ compared to those cells from patients without pulmonary hypertension and with secondary pulmonary hypertension (Yuan *et al*., 1998a, b). Taken together, these data suggest that K^+ channel dysfunction could lead to increased $[Ca^{2+}]_{cyt}$ and enhanced contraction and proliferation (Figure 4). Both ST- and bone morphogenetic protein (BMP)-induced apoptosis are diminished in PASMCs from IPAH patients compared to cells from patients with secondary pulmonary hypertension (Zhang *et al*., 2003), further suggesting a high level of $[K^+]_{cyt}$ that is protective against cell death. Lastly, sustained membrane depolarization, as may be seen with dysfunctional or inhibited K_V channels, leads to increases in $[Ca^{2+}]_{cyt}$ (Fleischmann *et al*., 1994), which can cause sustained PASMC contraction and promote PASMC proliferation. Taken together, these data suggest an inherent dysfunction in PASMC K_V channels from IPAH patients. As has already been discussed, dysfunctional K_V channels that decrease $I_{K(V)}$ would cause both membrane depolarization and an increase $[Ca^{2+}]_{cyt}$, and inhibit apoptotic cell shrinkage and cytoplasmic caspase activity. These altered ion equilibria can result in protection from apoptosis, persistent cell contraction and cellular proliferation, all of which would contribute to sustained pulmonary vasoconstriction and excessive pulmonary medial hypertrophy observed in IPAH.

Mutations in the BMP receptor type II (BMP-RII) gene have been linked to familial and sporadic PAH (Deng *et al*., 2000; Thomson *et al*., 2000; Janssen *et al*., 2002; Rindermann *et al*., 2003). A member of the TGF-β family of signalling molecules, BMP-RII is needed for recognition of all BMP ligands. Binding of BMP is followed by oligomerization of BMP-RII and RI. BMP receptor activation and the downstream signalling cascade it initiates via Smads and p38 can activate the transcription of genes needed to arrest cell growth and induce apoptosis (Itoh *et al*., 2000; Nohe *et al*., 2004). Although it is tempting to conclude that mutations in the receptor would lead to decreased rates of apoptosis in response to normal physiological signalling and thus contribute to medial hypertrophy observed in PAH, the exact

mechanism linking BMP-RII mutations and PAH is unknown. PASMCs expressing mutations of BMP-RII that are found in IPAH patients are resistant to BMP-induced apoptosis (Lagna *et al*., 2006). Furthermore, a link between BMP signalling and K^+ channel function has been established in PASMC. Treatment of normal PASMCs with BMP-2 or BMP-7 induces apoptosis by activation of caspases 3, 8 and 9, downregulation of anti-apoptotic Bcl-2 protein and cytochrome *c* release, whereas PASMCs from IPAH patients are resistant to BMP-induced apoptosis (Zhang *et al*., 2003; Lagna *et al*., 2006). Furthermore, $I_{K(V)}$ and protein levels of $K_V1.5$ were increased in normal PASMCs exposed to BMP-2 (Fantozzi *et al*., 2006; Young *et al*., 2006), providing a plausible link between dysfunctional BMP signalling, K^+ channel function and PASMC apoptosis.

Animal models of PAH have also been fundamental in elucidating the K^+/BMP/apoptosis axis in PAH. Upon expression of an inducible smooth muscle cell-targeted dominant-negative version of BMP-RII found in a PAH family, mice develop increased pulmonary arterial pressures, increased right to left ventricle + septum weight ratios and pulmonary artery muscularization without an effect on their systemic pressures (West *et al*., 2004), supporting a role for dysfunctional BMP signalling specifically in PAH pathogenesis. Furthermore, whole-lung tissue from these mice demonstrated decreased expression of $K_V1.1$, $K_V1.5$ and $K_V4.3$ mRNA as well as $K_V1.5$ protein (Young *et al*., 2006), echoing the reduced K_V transcripts found in human PASMCs from IPAH patients. Treatment of these PAH mice with nifedipine, a specific L-type Ca^{2+} channel blocker, reduced right systolic pressures, indicating a role for Ca^{2+} in disease onset (Young *et al*., 2006). Taken together, these studies suggest that a functional loss of BMP signalling in PASMCs can lead to vasoconstriction and remodelling through reduced $I_{K(V)}$, thereby contributing to the pathogenesis of PAH.

Exposure to chronic hypoxia (CH) has also been used as an animal model for PAH. Hypoxia is known to cause decreased $I_{K(V)}$ and K_V gene expression in PASMC (Yuan *et al*., 1993; Wang *et al*., 1997). In chronically hypoxic rats, dichloroacetate (DCA), a metabolic modulator that increases mitochondrial oxidative phosphorylation, was able to either reverse or prevent (depending on how soon after CH exposure it was given) haemodynamic abnormalities induced by CH, including the increase in pulmonary vascular resistance, right ventricular hypertrophy and PA remodelling (Michelakis *et al*., 2002). On a molecular level, DCA increased K^+ currents in PASMC and restored CH-induced $K_V2.1$ channel expression (Michelakis *et al*., 2002). DCA was also found to be effective in reversing monocrotaline (MCT)-induced PAH in rats, leading to similar recovery from haemodynamic symptoms as the CH rats (McMurtry *et al*., 2004). In the MCT study, it was found that DCA depolarized the mitochondria of PASMC, causing release of cytochrome *c*, and induced a significant increase in PASMC apoptosis and/or decrease in PASMC proliferation in the pulmonary arterial medial layer (McMurtry *et al*., 2004). Additionally, DCA rescued the MCT-induced decrease in $I_{K(V)}$, and protein and mRNA expression of $K_V1.5$ (McMurtry *et al*., 2004). Survivin, an inhibitor of apoptosis protein (Blanc-Brude *et al*., 2002), was found to be expressed in the pulmonary arteries of patients and rats with MCT-induced PAH, but not in patients or rats without MCT-induced PAH (McMurtry *et al*., 2005). In the rat model of PAH, administration of a dominant-negative form of survivin reversed PAH and improved survival, leading to many of the same haemodynamic improvements noted above, including decreased pulmonary vascular resistance, right ventricular hypertrophy and PA medial hypertrophy (McMurtry *et al*., 2005). Survivin treatment also led to increased $I_{K(V)}$, depolarized mitochondria which caused cytochrome *c* release and PASMC apoptosis (McMurtry *et al*., 2005). Given the above data, possible future therapeutic avenues for PAH may target K_V channels, leading to an increase in channel activity. Additionally, treatment with DCA or survivin inhibition should be explored further given their successes in reversing PAH and the haemodynamic sequelae in experimental rat models of PAH.

Development of future treatments that target K^+ channels will benefit from a careful examination of specific K^+ channel subtypes. In fully differentiated aortic smooth muscle cells, BK_{Ca} currents predominate, whereas in cells of the proliferative phenotype, IK_{Ca} currents are the dominant type (Neylon *et al*., 1999; Köhler *et al*., 2003; Jackson, 2005). It has been suggested that in vascular injury, a switch towards IK_{Ca} (from BK_{Ca}) may contribute to excessive intimal vascular smooth muscle cell proliferation (Köhler *et al*., 2003). Supporting this hypothesis, it was found that specific IK_{Ca} channel blockers significantly reduced intimal hyperplasia in a rat model of balloon catheter injury (Köhler *et al*., 2003). Although an exhaustive characterization of IK_{Ca} channels in PASMC from PAH patients has not been performed, it would be interesting to determine whether a similar phenotype is present in the vasculature of IPAH patients, thereby opening the possibility of new avenues of treatment.

PAH treatment: focus on K^+ channels and clinically approved PAH drug therapies and a look to the future

In the past decade, the number of clinically used drugs against PAH has increased dramatically. Most of these have been focused at the population of patients diagnosed with a severe form of PAH known as IPAH. Because of its complex aetiology, IPAH patients are now routinely treated with a combination of active drugs, such as prostanoid, phosphodiesterase, vascular endothelial growth factor receptor antagonists, statins and ET-1 receptor antagonists (Ghofrani *et al*., 2003; Hoeper *et al*., 2003, 2006; Martin *et al*., 2006; Souza *et al*., 2006; Puri *et al*., 2007). Targeting K^+ channels may have potential in the treatment of PAH, especially considering the fact that K^+ channel dysfunction is closely linked to many of the known contributing factors to the development of PAH: (a) enhanced K^+ channel activity limits membrane depolarization and Ca^{2+} influx via VDCC to cause pulmonary vasodilation (Yuan, 1995); (b) the phosphodiesterase 5 inhibitor sildenafil enhances cGMP-mediated activation of K_V and K_{Ca} channels in PASMC, causing vasodilation and improving pulmonary haemo-

dynamics (Michelakis *et al*., 2003); (c) BMP-2, a BMPR-II ligand, promotes PASMC proliferation by decreasing expression of anti-apoptotic Bcl-2 (Zhang *et al*., 2003) and by activating $I_{K(V)}$ in normal PASMC (Fantozzi *et al*., 2006); (d) pulmonary artery vasoconstriction by ET-1 is, in part, mediated by inhibiting K_V channel function (Shimoda *et al*., 2001); (e) iloprost (a stable analogue of prostacyclin) and prostacyclin activate multiple K^+ channels in vascular smooth muscle cells (Murphy and Brayden, 1995); (f) anorexigens inhibit K_V channel expression and function in PASMC (Wang *et al*., 1998; Perchenet *et al*., 2001); (g) anti-survivin therapy reverses the remodelling and haemodynamic effects of PAH, possibly due to the intermediate activation of K_V channels; and (h) *in vitro* gene transfection of *KCNA5* in PASMC causes membrane hyperpolarization and enhanced apoptosis (Brevnova *et al*., 2004), and (i) *in vivo* gene transfer of *KCNA5* in lung tissues reverses both vasoconstriction and pulmonary remodelling in chronically hypoxic rats (Pozeg *et al*., 2003). The latter represents the first successful example of K^+ channel gene therapy for a vascular disease. These studies clearly illustrate that targeting K^+ channels' subunit expression and/or function may be an important approach in treatment of PAH. The future holds much promise as far as the development of more effective anti-pulmonary hypertension drugs is concerned. Modulation of K^+ channel expression and function appears to be a common thread linking many current PAH therapies. Whether K^+ channels will become a focus in the future remains to be seen.

Conclusion

In summary, expression and function of membrane K^+ channels play important roles in regulating PASMC proliferation and apoptosis. In apoptosis, they mediate the K^+ efflux that is necessary for AVD. Additionally, K^+ efflux leading to decreased $[K^+]_{cyt}$ releases the inhibition of caspases, the final apoptotic mediators, which cleave proteins and DNA, leading to cell death. Furthermore, in PASMCs, K^+ channels are involved in proliferation, mostly through their control of the resting membrane potential. When K^+ channels close, the membrane depolarizes, leading to an influx of Ca^{2+}, an obligatory messenger for cell-cycle progression and proliferation. Dysfunctional K^+ channels have been implicated in the development of sustained pulmonary vasoconstriction and vascular medial hypertrophy associated with PAH. The three main classes of drugs used in the treatment of PAH are the prostacyclin analogues, endothelin receptor antagonists and phosphodiesterase 5 inhibitors; however, given the wide variation in individuals' responses to these medications, there is not a standardized treatment regimen for PAH. While the success of these drugs had greatly reduced the number of PAH patients receiving lung transplants in the last two decades, some patients fail to respond well even to different combinations of treatments. Therefore, K^+ channels as a therapeutic target for PAH remain an exciting possibility for future drug development.

Conflict of interest

The authors state no conflict of interest.

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