Mechanisms Controlling Vascular Tone in Pulmonary Arterial Hypertension: Implications for Vasodilator Therapy

Angel Cogolludo^a Laura Moreno^a Eduardo Villamor^b

^aDepartment of Pharmacology, School of Medicine, Universidad Complutense, Madrid, Spain;

^bDepartment of Pediatrics, University Hospital Maastricht, Research Institute Growth and Development (GROW), Maastricht, The Netherlands

Key Words

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Abstract

Pulmonary vasoconstriction is believed to be an early component of pulmonary arterial hypertension. Intracellular calcium concentration ($[Ca^{2+}]_i$) is a major trigger for pulmonary vasoconstriction; however, it is now well known that contractions and relaxations may also be elicited through Ca^{2+} -independent mechanisms. A variety of intracellular protein kinases and cyclic nucleotides have been identified as key determinants in controlling pulmonary vascular tone. Herein, we provide an overview of the main signaling pathways, which include protein kinase C, Rho kinases and cyclic nucleotides (cAMP and cGMP). This review also focuses on the role of store-operated Ca^{2+} channels and voltage-gated K⁺ channels, which are currently considered especially attractive in the pulmonary circulation and may represent new targets in the treatment of pulmonary arterial hypertension.

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Role of Vasoconstriction in the Physiopathology of Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a heterogeneous group of disorders characterized by a sustained increase in pulmonary artery pressure (PAP >25 mm Hg at rest or >30 mm Hg with exercise). The consequence of this increased right ventricle afterload is the failure of the afterload-intolerant right ventricle. PAH is a progressive disease of poor prognosis and is usually fatal within 3 years if left untreated. During the last few years, both genetic and mechanistic strategies have succeeded in identifying signaling pathways involved in PAH and new therapeutic agents have markedly improved physical function and have extended survival [1]. However, the pathogenesis of most forms of PAH is unknown and it is unclear whether the various types of PAH share a common pathogenic mechanism. Regardless of the initial trigger, three factors are thought to cause the increased pulmonary vascular resistance that characterizes this disease: vasoconstriction, remodeling of the pulmonary arteries (PA), and thrombosis in situ [1].

Thus, pulmonary vasoconstriction, due to an excess of vasoconstrictors or a decrease of vasodilators, is believed to be an early component of the pulmonary hypertensive process and recent findings even suggest that it may play a predominant role in PAH [2]. In fact, pharmacologic therapies for PAH are aimed at inducing pulmonary vascular smooth muscle relaxation and vasodilation [3]. One of the main concerns regarding the commonest vasodilator therapies in the treatment of PAH is the poor pulmonary selectivity and the high incidence of systemic side effects, which worsen the clinical situation. Therefore, the ideal vasodilator drug for the treatment of PAH should present selectivity for pulmonary over systemic vessels. Selective pulmonary vasodilation can be achieved through delivery of vasodilators directly to the lungs or targeting pulmonary specific processes. Thus, elucidation of the mechanisms controlling pulmonary vascular tone will contribute in our understanding of the physiology and the pathophysiology of pulmonary circulation, and could provide insights for new therapies, particularly in PAH. In this regard, much can be learned from hypoxic pulmonary vasoconstriction (HPV), a distinguishing feature of the small PA responsible for maintaining the ventilation-perfusion ratio during localized alveolar hypoxia. Therefore, the mechanisms involved in this phenomenon are very likely to be pulmonary selective.

As in the systemic circulation, the endothelium in the pulmonary circulation has a profound influence on vascular tone and remodeling [1, 4]. The endothelial cells release a variety of vasodilator (namely nitric oxide (NO), prostacyclin (PGI₂), and endothelium-derived hyperpolarizing factor) and vasoconstrictor (mainly endothelin-1 (ET-1) and thromboxane A₂ (TXA₂)) agents, as well as agents that affect the growth and the differentiation of vascular smooth muscle cells (VSMC). The role of the endothelium in the control of pulmonary vascular tone has been reviewed elsewhere [1, 4] and is beyond the scope of the present review. Apart from the endothelium, the control of pulmonary vascular tone involves a large number of factors acting through a wide variety of signaling pathways on many different targets within the pulmonary artery smooth muscle cells (PASMC). Herein, we provide an overview of the main signaling pathways controlling pulmonary vascular smooth muscle tone (i.e. pulmonary vasodilation and vasoconstriction). This review also focuses on the role of those ion channels especially attractive as possible targets in the pulmonary circulation.

Regulation of Pulmonary Vascular Tone

In either systemic or pulmonary vessels, contraction is finally determined by phosphorylation/dephosphorylation at Ser¹⁹ of the myosin light chain (MLC). MLC phosphorylation is mediated by the Ca²⁺-calmodulin-dependent MLC kinase (MLCK), which, in turn, is activated by the increase in intracellular calcium concentration ([Ca²⁺]_i). Therefore, [Ca²⁺]_i is a major trigger for pulmonary vasoconstriction; however, it is now well known that contractions and relaxations may also be elicited at a constant [Ca²⁺]_i, indicating that Ca²⁺ sensitization and desensitization of the contractile filaments also contribute to the control of pulmonary vascular tone. Taken together, the regulation of pulmonary vascular tone is finely regulated by changes in [Ca²⁺]_i and in Ca²⁺ sensitivity.

Ca²⁺-Homeostasis in PASMC. Increases in [Ca²⁺]_i are driven either through entry from extracellular space or by release from intracellular stores (fig. 1). Activation of L-type voltage-operated calcium channels (VOCC) is the main source of Ca²⁺ influx in VSMC. In addition, non-selective cation channels, encoded by the canonical transient receptor potential (TRPC) gene family, constitute the alternative pathways of Ca²⁺ entry in vascular myocytes. These channels have been reported to underlie the store (SOC)- and receptor (ROC)-operated Ca²⁺ influx [5], activated by depletion of Ca²⁺ from intracellular stores and agonists, respectively. The release of Ca²⁺ from the sarcoplasmic reticulum (SR) is mediated through channels gated by Ca²⁺ (caffeine/ryanodine) and inositol-1,4,5-trisphosphate (IP₃) and lead to a transient rise in [Ca²⁺]_i and contractile tension. On the other hand, Ca²⁺ extrusion and sequestration by SR occurs against ionic gradient and involves the activation of active transport systems including sarcoplasmic reticulum (SERCA) and plasmalemmal (PMCA) Ca²⁺ ATPases or facilitated diffusion systems such as Na⁺/Ca²⁺ exchanger.

Ca²⁺-Independent Mechanisms. An increase and a decrease in smooth muscle tension at a constant Ca²⁺ concentration are correspondingly referred to as Ca²⁺ sensitization and desensitization of smooth muscle contraction. Ca²⁺ sensitization is now known an important component of the constrictor response of many agonists and may involve a number of protein kinases. The RhoA and Rho kinase (ROCK) pathway is believed to be the most important modulator of Ca²⁺ sensitivity in smooth muscle, and a large number of vasoconstrictor agonists act through this pathway (see below: Rho Kinases (ROCKs)). Other protein kinases mediating Ca²⁺ sensi-

Fig. 1. Schematic overview of the mechanisms involved in the regulation of Ca^{2+} homeostasis in PASM. Agonists bind to their receptors triggering the synthesis of diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3). The former causes the activation of receptor-operated Ca^{2+} channels (ROC), whereas IP_3 stimulates Ca^{2+} release from sarcoplasmic reticulum (SR) through IP_3 channels. Depletion of SR causes the activation of store-operated Ca^{2+} channels (SOC) in order to refill the stores. L-type voltage-operated calcium channels (VOCC) are activated by membrane

depolarization (for instance due to inhibition of potassium channels) and make a marked contribution to the total concentration of Ca^{2+} in the cytosol. Ca^{2+} entry through VOCC may also trigger the activation of ryanodine receptors (RyR) present in SR, which respond releasing Ca^{2+} from the SR, a mechanism referred as Ca^{2+} -induced Ca^{2+} release (CICR). Finally, Ca^{2+} removal from the cytosol is triggered by sarcoplasmic reticulum (SERCA) and plasmalemmal (PMCA) Ca^{2+} ATPases or through facilitated diffusion systems such as $\text{Na}^+/\text{Ca}^{2+}$ exchanger.

zation include protein kinase C (PKC), mitogen-activated protein kinase, and non-receptor tyrosine kinases [6]. On the contrary, Ca^{2+} desensitization triggered by cyclic nucleotides (cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP)) may contribute to their relaxant effects.

Thus, in pulmonary and systemic vessels, tone is regulated by a variety of mechanisms acting through Ca^{2+} -dependent, Ca^{2+} -independent pathways, or both. However, the relative contribution of these mechanisms seems to differ substantially in the pulmonary versus systemic vessels. Therefore, we have highlighted those distinguishing mechanisms that could represent new targets in the treatment of PAH.

Protein Kinases Regulating Pulmonary Vascular Tone

The role of protein kinases in the control of pulmonary vascular reactivity has been extensively reviewed by Ward et al. [6]. In our review we describe the involvement of PKC and ROCK which are considered key signaling pathways in the pulmonary circulation.

Protein Kinase C

PKC is a widely distributed serine/threonine kinase that plays a key role in the regulation of many signal transduction mechanisms in response to a variety of stimuli [6]. PKC represents a family of several isoforms

that can be divided into conventional or cPKC (α , β I, β II, and γ), novel or nPKC (δ , ϵ , η , and θ), and atypical or aPKC (ζ and λ/ι) isoforms. The former group includes Ca^{2+} -dependent isoforms, whereas nPKC and aPKC are Ca^{2+} -independent. Unlike the other two groups, aPKCs do not respond to diacylglycerol (DAG). Several isoforms (α , β , δ , ϵ and ζ) seem to coexist in VSMC including PASMC [6, 7], and the specific isoform involved in any particular response may vary dependent on the agonist, species and vascular bed [6–9]. Upon stimulation, PKC is translocated from an inactive pool, usually the cytosol, to an active pool such as the plasma membrane, cytoskeleton or other particulate cellular component. The interaction of a PKC isoform with its protein substrate may trigger a cascade of protein kinases that ultimately stimulate vascular smooth muscle contraction. The pathways through which PKC mediates contraction include those involving alteration in $[\text{Ca}^{2+}]_i$ (directly via activation of Ca^{2+} channels or indirectly via inhibition of K^+ channels) and those Ca^{2+} -independent. The latter essentially involves the phosphorylation of CPI-17 which in turn inhibits MLC phosphatase (MLCP), increases MLC phosphorylation and enhances contraction.

Although the involvement of PKC on agonist-induced pulmonary vasoconstriction has largely been reported, many of these studies have been conducted with PKC modulators of dubious selectivity, limiting their conclusions. Molecular biology and genetic approaches and the currently available isoform-selective PKC inhibitors have made possible the elucidation of the involvement of specific PKC isoforms in cellular processes (such as vascular contractility) [for reviews, see 6, 10]. Unfortunately, only a few studies have determined the involvement of specific PKC isoforms in controlling pulmonary vascular tone. Thus, PKC δ has been suggested to play a role in angiotensin II-induced modulation of the contractile activity in PASMC [7]. We have reported that PKC ζ activation is involved in the voltage-gated K^+ (K_V) channel inhibition and vasoconstriction induced by TXA_2 in rat PA [9, 11]. Finally, it has been reported that HPV is markedly attenuated in PKC $\epsilon^{-/-}$ mice and that PKC ϵ appears to be an important determinant of susceptibility to chronic hypoxic PAH [12]. A better insight into the specific roles for individual isoforms of PKC may be important not only to elucidate the molecular basis of signal transduction, but also to identify new strategies for the treatment of PAH.

Rho Kinases (ROCKs)

ROCKs are serine/threonine kinases with a molecular mass of around 160 kDa. To date, two isoforms – ROCK-1 and ROCK-2 – have been identified. Although both are ubiquitously expressed, the latter is preferentially expressed in brain and skeletal muscle. Both ROCK-1 and ROCK-2 are expressed in vascular smooth muscle [13]. ROCKs are essentially distributed in the cytoplasm but are partially translocated to the membrane following activation by RhoA, a member of the Ras family of small GTP-binding proteins. Lipid messengers such as arachidonic acid or sphingosine phosphorylcholine are able to stimulate ROCK activity independently of RhoA. ROCKs have been found to regulate a wide range of fundamental cell functions such as motility, proliferation, apoptosis and contraction [13]. In fact, ROCKs are believed to be the most important modulators of Ca^{2+} sensitivity in smooth muscle [13, 14]. The main target for ROCKs is the 130-kDa myosin-binding subunit of MLCP. Phosphorylation of myosin-binding subunit by ROCKs leads to the inhibition of MLCP, which prevents MLC dephosphorylation and hence increases Ca^{2+} sensitivity and smooth muscle contractility [14]. In addition, ROCKs target other substrates that are important for smooth muscle contraction such as CPI-17, calponin or MLC [13]. Recently, it has been found that ROCKs can also be activated due to elevation of $[\text{Ca}^{2+}]_i$ [15]. Additional studies in this regard could shed light on the complex interaction between Ca^{2+} -dependent and -independent mechanisms.

Accumulating evidence indicates that ROCKs play an important role in controlling pulmonary vascular smooth muscle tone and in the pathogenesis of PAH. ROCKs and their activator RhoA are highly expressed in PA [2, 13] and have been reported to play a prominent role in pulmonary vasoconstriction induced by hypoxia [16] and a variety of agonists such as phenylephrine, prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$), TXA_2 and isoprostanes [6, 9, 17]. Moreover, several studies indicate that activation of the RhoA/ROCK pathway contributes to both vasoconstriction and vascular remodeling associated with several forms of PAH such as chronic hypoxia and monocrotaline-induced PAH [16, 18]. Support for the role of this pathway in PAH has been provided by ROCK inhibitors such as Y-27632 and fasudil. The poor pulmonary selectivity exhibited by either oral or intravenous administration of ROCK inhibitors is their major concern [18]. Interestingly, inhaled Y-27632 has been shown to be more effective than inhaled NO as a selective pulmonary vasodilator in hypoxic PAH [18]. Furthermore, this drug showed long-lasting effects (>5 h after a 5-min inhalation period). It

should be pointed out that most of these data have been obtained in animal models for PAH. In patients with severe PAH, Fukumoto et al. [19] have found that unlike oxygen inhalation, inhaled NO, or nifedipine, intravenous treatment with the ROCK inhibitor fasudil ameliorated pulmonary vascular resistance. The major limitation of this study is the inclusion of only 9 patients, but the results obtained suggest that ROCK inhibitors are very promising drugs in the treatment of PAH.

Cyclic Nucleotides Regulating Pulmonary Vascular Tone

cAMP

cAMP has been widely implicated in the control of pulmonary vascular tone [4]. Activation of multiple G-protein-coupled receptors (GPCRs), including β -adrenergic receptors and prostanoid receptors (EP and IP), can reduce vascular tone through stimulation of the enzyme adenylyl cyclase (AC), which leads to an increase in the production of cAMP. Therefore, AC plays a central role in the regulation of vascular tone and proliferation after cell surface GPCR activation. In rat PASMCs, the regulation of cAMP seems to be controlled mainly by AC2 (positively regulated by PKC), AC5 (negatively regulated by $G_i\alpha$ subunit, PKC and Ca^{2+} /CAM) and AC8 (positively regulated by Ca^{2+} /CAM) [20].

Agents such as forskolin or PGI_2 are thought to elicit their biological effects by increasing the intracellular levels of cAMP. Despite the extensive research, the precise mechanisms by which cAMP causes vascular relaxation remain unclear but it is generally accepted that cAMP may induce vascular relaxation by lowering $[Ca^{2+}]_i$ through inhibition of IP_3 formation (via inhibition of $PLC\beta$), inhibition of Ca^{2+} release from the SR, stimulation of Ca^{2+} uptake and/or extrusion, and inhibition of Ca^{2+} entry [4]. Similarly to cGMP, cAMP-elevating agents may induce smooth muscle hyperpolarization by modulation of K^+ channels. Indeed, cAMP-induced activation of calcium-activated (BK_{Ca}) and ATP-dependent (K_{ATP}) potassium channels has been largely described in PASMCs. However, recent evidence suggests the involvement of other types of potassium channels, such as TASK-1-related K^+ channels [21]. Nevertheless, cAMP can also regulate vascular tone through Ca^{2+} desensitization mechanisms, by increasing MLCP activity and by reducing MLCK, p42/p44 MAP kinase and Rho kinase activity [14]. Finally, recent studies pointed out that spatial organization of signaling pathways that regulate cAMP-

mediated effects may be cell-specific and play a key role in determining the effectiveness of this pathway [22]. Therefore, this compartmentalization of signaling components might add depth to our understanding of pulmonary specific processes and should be the objective of future studies.

Among the agents that induce an increase in cAMP levels, special attention has been paid to PGI_2 , the main product of arachidonic acid in the endothelial wall with potent vasodilator and antiaggregant actions. PGI_2 synthesis is decreased in endothelial cells from PAH patients, which seems to be due to a reduced expression of PGI_2 synthase [1]. Intravenous administration of PGI_2 (epoprostenol) causes clinic and hemodynamic improvements and prolongs survival in patients with PAH [3]. Indeed, PGI_2 and its analogues treprostinil (for continuous subcutaneous infusion), inhaled iloprost and the orally active beraprost have been an approved therapy for treating PAH in Europe, the USA or Japan [3]. Most recently, a novel mechanism of action of these PGI_2 -like therapies has been identified [23] which suggest that the nuclear receptor peroxisome proliferator-activated receptor β/δ may represent a novel therapeutic target for the treatment of PH.

NO/cGMP Pathway

NO is an endogenous vasodilator and inhibitor of smooth muscle cell proliferation, which exerts most of its physiological actions through the activation of soluble guanylyl cyclase (sGC) and the subsequent increase in cGMP levels. The increase in cGMP levels causes pulmonary vasodilation by mechanisms involving modulation of both Ca^{2+} homeostasis and sensitivity of contractile apparatus to $[Ca^{2+}]_i$ [4, 14].

Impaired NO synthesis, bioavailability, and/or activity has been described in several forms of PAH [1]. However, whether or not patients with PAH present altered expression of key proteins involved in the NO/cGMP pathway remains controversial and could be related to the type of PAH or the stage of the disease analyzed. Further studies have proposed a number of different mechanisms to explain alterations in the NO/cGMP pathway in PAH, including inactivation of either NO or sGC by oxidant stress [24]. Finally, exploring newly characterized mechanisms of modulation of sGC [25] might help us to identify pulmonary hypertensive derangements in the NO/cGMP pathway.

Inhaled NO is a selective and potent pulmonary vasodilator and an approved therapy for persistent pulmonary hypertension of the newborn. However, the clinical

use of inhaled NO in adults is limited by multiple factors, including the high rate of non-responders and unresolved toxicological questions such as rebound pulmonary hypertension in good responders [3], and novel therapeutic strategies in PAH aim at increasing NO-dependent, cGMP-mediated pulmonary vasodilation by activating sGC or by inhibiting the breakdown of cGMP by cyclic nucleotides phosphodiesterases (PDEs).

In the pulmonary vasculature, PDE5 represents the major metabolic pathway for cGMP and, therefore, a logical therapeutic target in PAH [24]. Further supporting the efficacy of PDE5 inhibitors in PAH, results from a randomized controlled study (conducted in 278 patients) showed continued benefit of sildenafil citrate [26] and led to its approval as an oral therapy for PAH in adults in the USA and Europe. Little is known, however, about the efficiency and security of sildenafil treatment in newborns and children. Although experimental data and recent case reports are courageous [27, 28], randomized controlled clinical trials are needed.

During the last years, new substances activating sGC have been identified. YC-1 was the first compound of this series to be identified [29]. YC-1 has been shown to decrease the dissociation rates of NO from the activated enzyme, sensitizing sGC towards NO and increasing the potency of NO by one order of magnitude [29]. Although it was initially characterized as a NO-independent activator of sGC, further studies suggest a synergistic effect of basal endothelium-derived NO and YC-1 on sGC or an YC-1-induced release of NO by endothelial cells, which remains to be determined [30]. BAY 41-2272 has shown to be direct activator of sGC through an allosteric, NO-independent regulatory site on sGC [29]. Accordingly, this drug induces vasorelaxation even in the absence of a functional endothelium and may also induce pulmonary relaxation by mechanisms independent of sGC. Administration of BAY 41-2272 has been shown to reverse experimental PAH [31]. Altogether, these results suggest that this drug may be an effective therapy for PAH and will soon enter clinical trials in pulmonary vascular disease [24].

Ion Channels in the Regulation of Pulmonary Vascular Tone

As in systemic vessels, ion channels play a crucial role in the regulation of pulmonary vascular tone. Among these, store-operated Ca^{2+} (SOC) and K_V channels are currently considered especially attractive in the pulmo-

nary circulation and may represent new targets in the treatment of PAH.

Store-Operated Ca^{2+} Channels

Two primary modes of Ca^{2+} influx exist in PASMC: voltage-dependent and -independent Ca^{2+} channels. The open probability of L-type VOCC is finely regulated by the activity of potassium channels (see below) which are the main contributors for membrane potential setting. Activation of VOCC is a key event during pulmonary and systemic vasoconstriction. Although VOCC antagonists, such as nifedipine, may be effective in lowering PAP, systemic vasodilation is their main limitation. Thus, chronically, administration of VOCC antagonists is beneficial in fewer than 10% of PAH patients who demonstrate acute vasoreactivity during testing [3].

In addition to VOCC, Ca^{2+} influx in PASMC can occur through voltage-independent channels which include both SOC and ROC. SOC are responsible for capacitative calcium entry (CCE) which has long been recognized as a major pathway for Ca^{2+} influx in non-excitabile cells. CCE is triggered by depletion of SR Ca^{2+} stores, which can result from either enhanced release of SR Ca^{2+} through ryanodine and IP_3 receptors in the SR membrane or depressed SR Ca^{2+} uptake by SERCA. CCE is thought to replete SR Ca^{2+} stores and signal cellular responses. The exact molecular identity of the proteins encoding SOC remains unclear, although isoforms in the TRPC subfamily, which are expressed in distal PA [32], are primary candidates. The role of CCE in PASMC has only very recently established. Thus, CCE has been shown to play an important role in agonist-mediated $[\text{Ca}^{2+}]_i$ elevation and pulmonary vasoconstriction. In addition, blockers of SOC, such as SKF-96365, nickel and La^{3+} , inhibit HPV [32], a specific phenomenon for distal PA. In support of this notion, CCE seems to have a more prominent role in small PA than in systemic arteries, which suggest that this mechanism may be of particular importance in regulating pulmonary vascular tone [33]. Finally, upregulation and functional enhancement of TRPC channels have been implicated in PASMC proliferation and overexpression of these channels has been reported in idiopathic [34] and hypoxic [35] PAH.

K_V Channels

Potassium channels, by regulating resting membrane potential, play an essential role in controlling vascular smooth muscle tone (fig. 2) [4, 36]. Among the different types of potassium channels identified in PASMC, there is increasing interest in K_V channels due to a number of

Fig. 2. K_V channels as a common target for vasoactive factors in pulmonary arteries. Vasoconstrictors (TXA_2 , 5-HT and ET-1) by activating different PKC isoforms cause K_V channel inhibition, which results in membrane depolarization, activation of VOCC, increase in intracellular Ca^{2+} and contraction. On the contrary, vasodilators may cause relaxation by activating K_V channels, which leads to the inhibition of VOCC, decrease in intracellular Ca^{2+} and vasodilation.

Table 1. Modulation of K_V channels in PASMC

Mediator	Effect	Mechanism	Tissue	Ref.
TxA_2	Inhibition	Atypical PKC (PKC ζ)	Rat Piglet	9 11
ET-1	Inhibition	cPKC	Human Rat	48 49
5-HT	Inhibition	cPKC/TyrK	Rat	8
Anorexigens	Inhibition	Direct?	Rat	39
Hypoxia	Inhibition	Direct? Redox mediator (ROS)?	Rat Dog	36 50
		$K_V1.5$	Rat PASMC transfected with hKCNA5 h $K_V1.5$ -transfected CHO cells	51 43
		$K_V2.1$	Rat	43
NO	Activation		Rat (primary culture)	52
cAMP	Activation		Rat PA	53

Effects and mechanism of action of different vasoactive mediators on the activity of K_V channels in PASMC isolated from different species.

facts. Firstly, they make a substantial contribution to whole-cell K^+ conductance and resting membrane potential in PASMC [36, 37]. Secondly, there is a large body of evidence indicating that these channels, particularly $K_{V1.5}$ and $K_{V2.1}$, are major effectors of HPV. Moreover, K_V channels are modulated by hypoxia and several mediators (table 1, fig. 2), indicating their prominent role as a common target for pulmonary vasoactive factors. Finally, decreased expression or function of K_V channels in PASMC has been involved in the pathogenesis of primary, hypoxic and anorexigen-induced PAH [37–39]. K_V channels exist as tetramers formed by four transmembrane $K_{V\alpha}$ subunits combined with modulatory cytosolic $K_{V\beta}$ subunits. In human PA, the expression of different $K_{V\alpha}$ and $K_{V\beta}$ subunits has been found [40]. Further diversity can be found in native channels because heterotetramers can be formed by the combination of distinct $K_{V\alpha}$ subunits. From the variety of K_V channels expressed in PASMC, special interest has been paid to $K_{V1.5}$, since decreased expression or activity and mutations of $K_{V1.5}$ occurs in human [37] and experimental [36, 38] primary and hypoxic PAH, and in vivo gene transfer of $K_{V1.5}$ reduces PAH and restores HPV [38]. Furthermore, decreased expression and function of K_V channels in PASMC leads to inhibition of apoptosis and promotes pulmonary vascular medial hypertrophy, whereas upregulation of $K_{V1.5}$ correlates with an increase in apoptosis/proliferation ratio and prevents and reverses PAH [41]. The reduction in K_V channels function and activity will result in a more depolarized membrane potential in PASMC from PAH patients and an increase in $[Ca^{2+}]_i$, leading to vasoconstriction and proliferation. On the contrary, normalization of elevated pulmonary vascular resistance in different models of PAH following treatment with dichloroacetate (an inhibitor of the mitochondria enzyme pyruvate dehydrogenase kinase) and survivin-targeting gene therapy has been associated with the restoration and/or activation of K_V channels [41, 42]. Altogether, these data support the idea that gene transfer of K_V channels or drugs activating K_V channels or preventing its inhibition may be a novel therapeutic strategy in the management of PAH.

Additional Considerations

Searching for more effective and safer treatments specific for PAH would depend on our ability to integrate the available and incoming information. Indeed, growing evidence suggests that the relative contribution of the mechanisms

described above may depend on a wide number of factors related to experimental and clinical conditions, including differences between species, age, vascular diameter, different experimental models of PAH and different stages of the disease.

Firstly, different responses to vasoconstrictor and vasodilator stimuli can be found along the pulmonary tree, indicating changes in the mechanisms involved in their responses. For instance, the stronger HPV in small sized as compared to conduit PA has been related to a preferential expression of the O_2 -sensitive K_V channels $K_{V1.5}$ and $K_{V2.1}$ [43]. These results suggested that K_V channels activity could determine the segmental heterogeneity of the hypoxia-mediated pulmonary responses.

In children, PAH is a devastating disease with a worse prognosis than in adults. Particularly, the neonatal period appears to be of particular interest in terms of the relative contribution of the mechanisms described above, because during this period the pulmonary circulation undergoes an exceptional process of reduction of pulmonary vascular resistance and is particularly vulnerable to develop PAH in response to insults such as hypoxia. In addition, many different congenital heart defects are associated with increased risk for the development of PAH. The normal postnatal decrease of PAP has been correlated with an increased responsiveness to the NO/cGMP pathway in numerous species [44, 45]. Developmental changes in the expression and activity of a variety of proteins such as cytosolic superoxide dismutase [44], sGC [30] and PDE5 [28] have been implicated in this phenomenon. Moreover, the relative efficacy of a vasodilator may be intimately associated with maturational changes in the mechanisms underlying pulmonary vasoconstriction. Thus, the TXA_2 -evoked contraction, which shifts from a Ca^{2+} -dependent to a Ca^{2+} -independent pattern during postnatal maturation, determines the efficacy of a given vasodilator pathway [11] (fig. 3).

Finally, the role of a given pathway may vary depending on the phenotypic state of pulmonary arterial smooth muscle. Thus, Barman et al. [46] reported that PKC activation inhibited BK_{Ca} channel activity in hypertensive PASMC, which is opposite to that observed in normotensive PASMC, whereby pharmacologic activation of specific PKC isozymes opened BK_{Ca} channels [47].

In summary: The control of pulmonary vascular tone involves the activation of a wide variety of signaling cascades modulating Ca^{2+} homeostasis, Ca^{2+} sensitivity or both. There is now good evidence to indicate that ion channels, particularly SOC and K_V , are key targets for

Fig. 3. Changes in the mechanisms leading to vasoconstriction during postnatal maturation determine vasodilator efficacy. **a** Vasoconstriction induced by the TXA₂ analog U46619 shifts from a nifedipine-dependent to a nifedipine-independent pattern in 1-day vs. 2-week-old piglet PA, respectively. **b** Effects of different vasodilator agents on the vasoconstriction and the increase in [Ca²⁺]_i induced by U46619 in 2-week-old piglet PA. Concentration-dependent effects of NO, nifedipine, forskolin and Y-27632 on [Ca²⁺]_i (upper panels) and contractile force (lower panels), respectively. **c** [Ca²⁺]_i-force relationship for the effects of these vasodilator agents. Data show mean $\pm$ SEM (n = 4–6). Adapted from Cogolludo et al. [9]; reprinted with permission.

vasoactive factors in the pulmonary circulation. Importantly, the relative contribution of all these mechanisms in controlling pulmonary vascular reactivity may vary according to different situations, which raise important issues regarding the choice of the adequate vasodilator to treat PAH. In this regard, an emerging option for the treatment of PAH is the combined use of drugs with different mechanisms of action [3]. Research in this field should focus not only on the discovery of new targets, but also on the possible interactions of the main pathophysiologic pathways in the pulmonary vasculature.

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