New mechanisms of pulmonary arterial hypertension: role of Ca\textsuperscript{2+} signaling

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Kuhr FK, Smith KA, Song MY, Levitan I, Yuan JX. New mechanisms of pulmonary arterial hypertension: role of Ca\textsuperscript{2+} signaling. Am J Physiol Heart Circ Physiol 302: H1546–H1562, 2012. First published January 13, 2012; doi:10.1152/ajpheart.00944.2011.—Pulmonary arterial hypertension (PAH) is a severe and progressive disease that usually culminates in right heart failure and death if left untreated. Although there have been substantial improvements in our understanding and significant advances in the management of this disease, there is a grim prognosis for patients in the advanced stages of PAH. A major cause of PAH is increased pulmonary vascular resistance, which results from sustained vasoconstriction, excessive pulmonary vascular remodeling, in situ thrombosis, and increased pulmonary vascular stiffness. In addition to other signal transduction pathways, Ca\textsuperscript{2+} signaling in pulmonary artery smooth muscle cells (PASMCs) plays a central role in the development and progression of PAH because of its involvement in both vasoconstriction, through its pivotal effect of PASMC contraction, and vascular remodeling, through its stimulatory effect on PASMC proliferation. Altered expression, function, and regulation of ion channels and transporters in PASMCs contribute to an increased cytosolic Ca\textsuperscript{2+} concentration and enhanced Ca\textsuperscript{2+} signaling in patients with PAH. This review will focus on the potential pathogenic role of Ca\textsuperscript{2+} mobilization, regulation, and signaling in the development and progression of PAH.

vessel remodeling; vasoconstriction; calcium regulation; calcium channel; canonical transient receptor potential channel

results in right ventricular hypertrophy and eventually right heart failure and death.

Pulmonary arterial hypertension (PAH) is a disease that is rarely diagnosed during routine medical examinations. Instead, it is typically made from exclusion of other disorders such as congenital heart disease, emphysema, or pulmonary embolism. Although a noninvasive estimation of pulmonary arterial pressure (PAP) can be derived through echocardiography, there are limitations that make screening and diagnosis challenging (123). The gold standard for clinical diagnosis of pulmonary hypertension is by right heart catheterization. The mean PAP (mPAP) for a healthy adult is 10–20 mmHg, whereas mean systemic arterial pressure is 70–105 mmHg. Pulmonary hypertension is defined clinically as a mPAP ≥ 25 mmHg at rest or a mPAP ≥ 30 mmHg during exercise (6). Importantly, PAH can be distinguished from other forms of pulmonary hypertension with the additional criterion of a pulmonary wedge pressure ≤ 15 mmHg (6). Table 1 shows the clinical classifications set forth by the most recent World Symposium on Pulmonary Hypertension held in Dana Point, CA, in 2008. These conferences were instrumental in assigning categories based on shared underlying pathologies that allowed investigators to focus treatments on a well-defined group of patients. There are two widely used functional classification systems used by physicians for assessing patients with pulmonary hypertension: the New York Heart Association system and the World

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Introduction

The lungs are the only organ that receives the entire cardiac output (CO), so the pulmonary circulation is maintained in a high-flow, low-pressure, and low-resistance state under normal physiological conditions. The human pulmonary vasculature is composed of pulmonary arteries, precapillary arterioles, capillaries, and pulmonary veins. The walls of pulmonary arteries and arterioles are thin, and the myogenic tone in pulmonary arteries is small compared with systemic arteries (e.g., in mesenteric, coronary, renal, and cerebral arteries). Therefore, pulmonary arterial distension and recruitment (opening of closed blood vessels) are two important mechanisms for reducing pulmonary vascular resistance (PVR) when blood flow or CO is increased (e.g., during heavy exercise). Under pathological conditions, however, increased PVR is a major cause for the development of pulmonary hypertension. Elevated PVR increases the right heart afterload and over time...
Health Organization system. Table 2 lists the four classes of functional assessment for each system with a brief description (98). Recent epidemiological data from the Registry to Evaluate Early And Long-term pulmonary arterial hypertension disease management (REVEAL) study conducted from March 2006 to September 2007 in the United States reported that the most common age of patients with a diagnosis of PAH is between 45 and 54 years, with a mean age of 44.9 years. Additionally, PAH is more likely to affect women by a 3.6-to-1 ratio (7, 40). These results closely matched the French registry study with age and severity at time of diagnosis. However, the REVEAL data showed a preponderance of women and these patients had a tendency to be obese, whereas French patients with PAH were associated with human immunodeficiency virus (10, 40, 49).

In humans, there are 15 orders of pulmonary arteries between the main pulmonary artery and the capillaries with 15 orders of pulmonary veins between the capillaries and the left atrium (Fig. 1, A and B) (47, 68). The pulmonary artery is composed of three layers: intima (endothelial cells), media (smooth muscle cells), and adventitia (fibroblasts) (Fig. 1 D).

Table 1. WHO Classification of Pulmonary Hypertension: Dana Point, 2008

<table>
<thead>
<tr>
<th>1. Pulmonary arterial hypertension</th>
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<tr>
<td>1.1 Idiopathic PAH</td>
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<td>1.2 Heritable</td>
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<tr>
<td>1.2.1 BMPR2</td>
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<td>1.2.2 ALK1, endoglin (with or without hereditary hemorrhagic telangiectasia)</td>
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<tr>
<td>1.2.3 Unknown</td>
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<tr>
<td>1.2.4 Drug and toxin induced</td>
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<tr>
<td>1.2.5 Associated with</td>
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<tr>
<td>1.2.5.1 Connective tissue disease</td>
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<td>1.2.5.2 HIV infection</td>
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<td>1.2.5.3 Portal hypertension</td>
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<td>1.2.5.4 Congenital heart diseases</td>
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<td>1.2.5.5 Schistosomiasis</td>
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<td>1.2.5.6 Choroid hemolytic anemia</td>
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<td>1.5 Persistent pulmonary hypertension of the newborn</td>
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<tr>
<td>1.6 Pulmonary veno-occlusive disease (PVOD) and/or pulmonary capillary hemangiomatosis (PCH)</td>
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2. Pulmonary hypertension due to left heart disease

2.1 Systolic dysfunction

2.2 Diastolic dysfunction

2.3 Valvular disease

3. Pulmonary hypertension due to lung diseases and/or hypoxia

3.1 Chronic obstructive pulmonary disease

3.2 Interstitial lung disease

3.3 Other pulmonary diseases with mixed restrictive and obstructive pattern

3.4 Sleep-disordered breathing

3.5 Alveolar hypoventilation disorders

3.6 Chronic exposure to high altitude

3.7 Developmental abnormalities

4. Chronic thromboembolic pulmonary hypertension (CTEPH)

5. PH with unclear multifactorial mechanisms

5.1 Hematologic disorders: myeloproliferative disorders, splenectomy

5.2 Systemic disorders: sarcoidosis, pulmonary Langerhans cell histiocytosis, lymphangioleiomyomatosis, neurofibromatosis, vasculitis

5.3 Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders

5.4 Others: tumoral obstruction, fibrosing mediastinitis, chronic renal failure (on dialysis)

WHO, World Health Organization; PAH, pulmonary arterial hypertension; BMPR2, bone morphogenetic protein receptor 2. Reprinted from Simonneau et al. (104) with permission.

Table 2. Functional Classification of Pulmonary Hypertension

<table>
<thead>
<tr>
<th>A. New York Heart Association functional classification</th>
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<tr>
<td>Class 1: No symptoms with ordinary physical activity.</td>
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<tr>
<td>Class 2: Symptoms with ordinary activity. Slight limitation of activity.</td>
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<tr>
<td>Class 3: Symptoms with less than ordinary activity. Marked limitation of activity.</td>
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<td>Class 4: Symptoms with any activity or even at rest.</td>
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<th>B. WHO functional assessment classification</th>
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<tr>
<td>Class I: Patients with PH but without resulting limitation of physical activity. Ordinary physical activity does not cause undue dyspnea or fatigue, chest pain, or near syncope.</td>
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<tr>
<td>Class II: Patients with PH resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity causes undue dyspnea or fatigue, chest pain, or near syncope.</td>
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<tr>
<td>Class III: Patients with PH resulting in marked limitation of physical activity. There are comfortable at rest. Less than ordinary activity causes undue dyspnea or fatigue, chest pain, or near syncope.</td>
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<tr>
<td>Class IV: Patients with PH with inability to carry out any physical activity without symptoms. These patients manifest signs of right heart failure. Dyspnea and/or fatigue may even be present at rest. Discomfort is increase by any physical activity.</td>
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PH, pulmonary hypertension. Rubin et al. (98) with permission from the American College of Chest Physicians.
decreases exponentially from orders 1 to 15 with an exponential increase in pulmonary artery branches (Fig. 1C). This leaves a total area of approximately 25% for large conducting arteries (diameter <0.6 mm), 44% for medium-sized (0.2–0.6 mm) and 30% for small vessels (diameter <0.2 mm). Although all branches of the pulmonary artery are involved in determining the level of PVR, changes in medium and small pulmonary arteries contribute more to the alteration of PVR in animals and patients with pulmonary hypertension.

PAP is a function of CO and PVR: \( PAP = CO \times PVR \). PVR is inversely proportional to the intraluminal radius \( (r) \) of pulmonary arteries (PVR \( \propto 1/r^4 \)) (Fig. 1C). An important highlight of this equation is that a small change in the radius of a pulmonary blood vessel can have a dramatic impact on PVR since the radius is raised to the fourth power. As shown in Fig. 2, a small amount of pulmonary vasoconstriction induced by hypoxia, or an 18% decrease in the diameter of the medium or small pulmonary arteries, would cause a significant increase in mean PAP (from 16 to 39 mmHg or from 24 to 59 mmHg). Given the relationship between PVR and pulmonary artery intraluminal radius or diameter, total PVR can be significantly altered by small changes in contraction by these arteries or in the wall thickness of these arteries.

Pathogenic Mechanisms of PAH

Regardless of the initial genetic or pathogenic trigger(s), the pathogenesis of PAH can be attributed to the combined effects of sustained vasoconstriction, concentric vascular remodeling, in situ thrombosis, and arterial wall stiffening, resulting in elevated PVR (Fig. 3) (75, 136). As shown in the angiogram (Fig. 3A), there is a striking contrast in the pulmonary vasculature of a normal subject versus a patient with PAH, which shows a significant decrease in the number of small vessels present. All patients with PAH share common pathological features such as precapillary arteriopathy; increased thickness of the intima, media and adventitia of peripheral arteries; muscularization of the precapillary arterioles and capillaries; and obliteration of small vessels (Fig. 3, A and C) (34, 112, 131).

The vasoconstriction induced by acute hypoxia, or hypoxic pulmonary vasoconstriction, is important to optimize ventilation-perfusion matching, diverting blood from poorly ventilated areas of the lung to the regions that are better ventilated for more efficient gas exchange (126, 127). However, persistent hypoxia causes pulmonary hypertension in animals and patients by inducing sustained pulmonary vasoconstriction and pulmonary vascular medial hypertrophy. Although PAH is disparate from hypoxia-induced pulmonary hypertension, sustained vasoconstriction and excessive pulmonary vascular remodeling are present in both forms of pulmonary hypertension.

The thickness and tissue mass of the pulmonary arterial walls are maintained by a balance between cell proliferation and apoptosis. A disruption of this balance in favor of proliferation can lead to thickening of the wall, narrowing, and eventually obliteration of the vessel lumen. Pulmonary vascular remodeling refers to the structural changes that lead to hypertrophy and/or luminal occlusion (33, 111). An increase in cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)\(_{cyt}\)] in pulmonary artery smooth muscle cells (PASMCs) is a major trigger for pulmonary vasoconstriction, as well as an important stimulus for cell proliferation and migration, two major causes for pulmonary vascular remodeling. Thrombotic lesions are also often appar-

Fig. 1. Structure and architecture of the pulmonary vasculature. A: pulmonary angiogram of the human lung showing the structure of the vasculature tree from large conducting vessels down to higher generation branches (arterioles). B: scheme of the pulmonary vasculature depicts the 15 orders of pulmonary arteries and veins with capillaries in between. C: cross section of pulmonary artery showing a small decrease in radius \( (r) \) or diameter can lead to a significant increase in pulmonary vascular resistance (PVR) and, as a result, elevation of pulmonary arterial pressure (PAP). D: schematic demonstrating the 3-layer structure of an artery from the lumen to the outside of the vessel. PA, pulmonary artery; PV, pulmonary vein; CO, cardiac output.
Ca\textsuperscript{2+} Is Required for Pulmonary Vasoconstriction and PASMC Proliferation

Both sustained pulmonary vasoconstriction and vascular remodeling are directly mediated by PASMC contraction and proliferation, respectively. Increased [Ca\textsuperscript{2+}]\textsubscript{cyt} is a major stimulus for cellular proliferation (Fig. 5). Both nuclear and cytosolic Ca\textsuperscript{2+} pools promote proliferation by activating Ca\textsuperscript{2+}-dependent kinases (e.g., CaMK), immediate early genes, and other transcription factors [e.g., c-Fos, nuclear factor of activated T-cells (NFAT), cAMP response element binding protein (CREB)], which are necessary for cell growth (Fig. 5A) (5, 11, 43, 102). Increases in cytoplasmic and nuclear [Ca\textsuperscript{2+}] trigger Ca\textsuperscript{2+}-dependent gene transcription in vascular smooth muscle cells (32, 45, 46, 76). Ca\textsuperscript{2+} can also affect gene expression through its interaction with protein kinase C (PKC) and calmodulin (CaM) or by activation of the proteins involved in the cell cycle (cyclins and cyclin dependent kinases). In addition to the stimulation of quiescent cells to enter the cell cycle (G\textsubscript{0} to G\textsubscript{1} transition), Ca\textsuperscript{2+} or Ca\textsuperscript{2+}/CaM is also required for progression from G\textsubscript{1} to S, from G\textsubscript{2} to mitosis checkpoints, and through mitosis (Fig. 5A) (11, 23, 30, 56, 73, 103). In PASMCs specifically, both increased [Ca\textsuperscript{2+}]\textsubscript{cyt} and intracellularly stored Ca\textsuperscript{2+} are essential for proliferation (42). In the presence of serum and growth factors, the removal of extracellular Ca\textsuperscript{2+} and the depletion of intracellularly stored Ca\textsuperscript{2+} inhibit proliferation of PASMCs, demonstrating the requirement for Ca\textsuperscript{2+} for the cell cycle progression and cell growth (Fig. 5C) (42).

In addition to stimulating proliferation, increased [Ca\textsuperscript{2+}]\textsubscript{cyt} is necessary for contraction in PASMCs (Fig. 5A) (14, 17, 29, 70, 74, 77). Both Ca\textsuperscript{2+} influx through plasma membrane Ca\textsuperscript{2+} channels and Ca\textsuperscript{2+} release from intracellular stores [e.g., the sarcoplasmic reticulum (SR)] contribute to a rise in [Ca\textsuperscript{2+}]\textsubscript{cyt}. In rat pulmonary arterial rings, the removal of extracellular Ca\textsuperscript{2+} prevents high K\textsuperscript{+}-induced contraction (70), indicating that Ca\textsuperscript{2+} influx through plasmalemmal channels is necessary for contraction (Fig. 5B). When [Ca\textsuperscript{2+}]\textsubscript{cyt} increases, it binds to CaM, which then activates myosin light chain kinase. Activated myosin light chain kinase phosphorylates the regulatory light chain of myosin, allowing for the activation of myosin ATPase. The ensuing hydrolysis of ATP provides the energy source needed for the cross-bridging cycles between myosin and actin filaments. These cross-bridging interactions constitute cellular contraction (106, 107) and, in the case of concerted contraction of PASMCs, pulmonary vasoconstriction. Sustained pulmonary vasoconstriction is thought to be partly responsible for the elevated PVR and PAP observed in some patients with IPAH. Thus a rise in [Ca\textsuperscript{2+}]\textsubscript{cyt} in PASMCs due to Ca\textsuperscript{2+} influx through various Ca\textsuperscript{2+}-permeable channels in the plasma membrane is required for agonist-induced pulmonary vasoconstriction and for serum and growth factor-mediated PASMC proliferation (which leads to pulmonary vascular medial hypertrophy and pulmonary vascular remodeling).

Regulation of [Ca\textsuperscript{2+}]\textsubscript{cyt} in Normal PASMCs: Two Mechanisms

Voltage-dependent Ca\textsuperscript{2+} influx pathway. The membrane potential (E\textsubscript{m}) in pulmonary vascular smooth muscle cells depends on Na\textsuperscript{+}, K\textsuperscript{+}, and Cl\textsuperscript{−} concentration gradient across the plasma membrane and the relative ion permeabilities (P). The Goldman-Hodgkin-Katz voltage equation, or the Goldman equation, is used to determine the equilibrium potential across
a cell membrane taking into account all of the ions that are permeable through the membrane. In resting vascular smooth muscle cells, $E_{\text{m}}$ is controlled primarily by $K^{+}$ permeability and gradient, because $P_K$ is much greater than $P_{\text{Cl}}$, $P_{\text{Na}}$, and $P_{\text{Ca}}$ in excitable cells and many types of nonexcitable cells.

PASMCs maintain a negative $E_{\text{m}}$, which is -40 to -50 mV in cultured and freshly dissociated PASMCs (measured by the patch-clamp technique) (3, 4, 138) and -50 to -65 mV in PASMCs of rat pulmonary arteries (measured by intracellular electrode) (114, 115). Although the $E_{\text{m}}$ in PASMCs is close to the calculated equilibrium potential for $K^{+}$ ($E_K$, approximately -85 mV, based on the Nernst equation), the 20–40-mV difference between the resting $E_{\text{m}}$ and $E_K$ indicates that, in addition to $K^{+}$, the plasma membrane of PASMCs may be permeable to other cations (e.g., Na$^+$, Ca$^{2+}$, H$^+$, Mg$^{2+}$) and anions (e.g., Cl$^-$, HCO$_3^-$). In other words, the cation and anion currents through membrane channels other than $K^{+}$ channels may also contribute to the regulation of the resting $E_{\text{m}}$ in PASMCs.

$K^{+}$ permeability is directly related to the whole cell $K^{+}$ current ($I_K$), which is determined by the following equation: $I_K = N \times i \times P_{\text{open}}$, where $N$ is the number of membrane $K^{+}$ channels, $i$ is the amplitude of the single-channel $K^{+}$ current, and $P_{\text{open}}$ is the steady-state probability that the $K^{+}$ channel is open. The activity of $K^{+}$ channels in the membrane is thus important for the regulation of $E_{\text{m}}$ and plays a role in vascular contractility. Voltage-gated $K^{+}$ ($K_v$) channels, the most diverse group of $K^{+}$ channels, are ubiquitously expressed in vascular smooth muscle cells (22, 87). When $K_v$ channels close, the membrane depolarizes, which leads to increased $[\text{Ca}^{2+}]_{\text{cyt}}$ by inducing $\text{Ca}^{2+}$ influx through voltage-dependent $\text{Ca}^{2+}$ channels (VDCC) (Fig. 6A). Inhibition of $K_v$ channels with 4-aminopyridine reduces whole cell $K^{+}$ currents (Fig. 6B), causes membrane depolarization (Fig. 6B,b), and results in increased $[\text{Ca}^{2+}]_{\text{cyt}}$ in PASMCs. In isolated pulmonary arterial rings, inhibition of $K_v$ channels by 4-aminopyridine increases isometric tension as a result of PASMC contraction and vasoconstriction in response to membrane depolarization and $\text{Ca}^{2+}$ influx through VDCC (Fig. 6B,d).

VDCC can be classified into six different subtypes based on their functional characteristics (39, 119, 120). However, in PASMCs L- and T-type channels are the important channels for voltage-gated $\text{Ca}^{2+}$ entry involved in excitation-contraction coupling and cell proliferation (36, 61). The L-type VDCC are

![Fig. 3. Major causes for the elevated PVR in patients with pulmonary arterial hypertension (PAH): the effects of remodeling and corresponding changes in PVR. A: a significant loss of the small vessels of the pulmonary vasculature can be seen in an angiogram from a healthy (normal) patient versus a patient with PAH. Histological cross sections of small arteries reveal the extensive hypertrophy of the adventitia, media, and intima (or concentric pulmonary arterial remodeling) that occurs in patients with PAH [adapted by permission from BMJ Publishing Group Limited. Bedford et al. (8)]. B: list of the major causes leading to an increase PVR in patients with PAH. C: small arteries from a normal subject and 2 different patients with PAH show the change in architecture [adventitial and medial hypertrophy, intimal lesion, and intraluminal obliteration (top), as well as significant medial hypertrophy (bottom)] that occurs in the pulmonary vasculature causing elevated PVR. PASMC, pulmonary artery smooth muscle cell; PAEC, pulmonary artery endothelial cells.](http://ajpheart.physiology.org/issue)
activated by high voltage, whereas inactivation is slow. The T-type channels are activated by low voltage, whereas inactivation is much faster than L-type channels. The activation threshold for L-type channels is approximately \(\pm 30 \text{ to } \pm 20 \text{ mV}\), and the maximal activation often occurs at \(\pm 10 \text{ to } \pm 15 \text{ mV}\). For T-type VDCC, the activation threshold and the maximal activation both shift to the left, with the former at approximately \(-40 \text{ to } -30 \text{ mV}\) and the latter at about \(-10 \text{ mV}\) (35). Both types of VDCC are thought to exist in three states: resting (or closed), open, and inactivated conformations. Switching conformations from the resting or closed state to the open state is dependent on membrane depolarization (77). Ca\(^{2+}\) entry through T-type channels is linked to cell proliferation with the channel activity associated with G1-S boundary of cell-cycle progression in rat aortic smooth muscle cells (29, 78). It has been reported that T-type channels are needed in the early stages of skeletal muscle and cardiac development. Expression of T-type channels is lost as smooth muscle cells differentiate and lose their ability to proliferate; expression and function of T-type channels reappear in pathological cell proliferation in cancer cells and in cultured vascular smooth muscle cells (29). The L-type channel can be targeted indirectly through G protein-coupled receptors (GPCRs) by a host of cellular second messenger systems that are activated by various agonists including, but not limited to, norepinephrine, endothelin, angiotensin II, and 5-HT. Stimulation by protein kinases, such as protein kinase G, and nitric oxide can inhibit

**Fig. 4.** Potential pathogenic mechanisms involved in PAH. Flow chart demonstrating the 4 major causes of elevated PVR and how they lead to PAH and eventually right heart failure.

**Fig. 5.** An increase in cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{cyt}\)) in PASMCs is required for pulmonary vasoconstriction and plays an important role in cell proliferation and vascular remodeling. A: when the [Ca\(^{2+}\)]\(_{cyt}\) rises because of Ca\(^{2+}\) influx through different Ca\(^{2+}\) channels in the plasma membrane and Ca\(^{2+}\) mobilization from the intracellular stores [e.g., sarcoplasmic reticulum/endoplasmic reticulum (SR/ER)], Ca\(^{2+}\) binds calmodulin (CaM) which causes PASMC contraction by activating (via phosphorylation) myosin light chain (MLC) kinase (MLCK). Increased [Ca\(^{2+}\)]\(_{cyt}\) also activates CaM kinase (CaMK) and mitogen-activated protein kinase (MAPK), as well as other transcription factors [nuclear factor of activated T cells (NFAT), CREB response element binding protein (CREB), activator protein-1 (AP-1), and NF-xB], to stimulate PASMC proliferation by propelling Ca\(^{2+}\)-sensitive steps in the cell cycle progression. B: removal of extracellular Ca\(^{2+}\) (0 Ca) significantly inhibits vasoconstriction (determined by active tension) induced by 40 mM K\(^+\) (40 K) and phenylephrine (PE) in isolated rat pulmonary arterial rings (70). C: rat PASMC growth is significantly inhibited by chelation of extracellular Ca\(^{2+}\) with EGTA or by valinomycin (Val)-induced increase in K\(^+\) efflux (88). Cells are cultured in serum- and growth factor-contained media for 6 days in the absence of EGTA or Val (Cont), and the presence of 100 \(\mu\)M Val or 2 mM EGTA. MLCP, myosin light chain phosphatase; PAFB, pulmonary arterial fibroblasts; P, phosphorylated; G, M, S, phases of the cell cycle. **\(P < 0.01\); ***\(P < 0.001\).
L-type channels, whereas protein kinase C (PKC) has been shown to potentiate L-type currents (25, 57). There is also evidence that nonreceptor tyrosine kinases, such as c-Src, enhance L-type currents (128).

Receptor- and store-operated Ca$^{2+}$/H$^{+}$ influx pathways. Stimulation of membrane receptors, such as GPCRs and receptor tyrosine kinases (RTKs), by their extracellular ligands results in the activation of phospholipase C and the production of two important second messengers, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP$_3$). DAG can then open receptor-operated Ca$^{2+}$/H$^{+}$ channels (ROC), leading to Ca$^{2+}$/H$^{+}$ influx and increased [Ca$^{2+}$]$_{cyt}$ (Fig. 7A). This process is referred to as receptor-operated Ca$^{2+}$/H$^{+}$ entry (ROCE). Additionally, IP$_3$ stimulates the IP$_3$ receptor (IP$_3$R), which is a Ca$^{2+}$ release channel on the sarcoplasmic reticulum/endoplasmic reticulum (SR/ER) membrane, to release Ca$^{2+}$ from the SR/ER to the cytosol. This leads to a depletion or a significant reduction of the SR/ER Ca$^{2+}$ store. Upon depletion of Ca$^{2+}$ from the SR/ER, a Ca$^{2+}$ deficiency signal is transmitted to store-operated Ca$^{2+}$ channels (SOC) on the plasma membrane causing SOC to open and allow Ca$^{2+}$ to flow into the cytosol, this process is referred to as store-operated Ca$^{2+}$ entry (SOCE). The cytosolic Ca$^{2+}$ is then sequestered into the SR/ER by the sarco(endo)plasmic reticulum Ca$^{2+}$/ATPase (SERCA), thus replenishing Ca$^{2+}$ stores (Fig. 7B) (12, 24, 92). The exact cellular and molecular mechanisms of SOCE have been the research focus of many investigators since Putney et al. (93) first described SOCE, referred to then as capacitative Ca$^{2+}$ entry. Although it is still controversial, many agree that the stromal interacting molecule (STIM) and Orai channels, as well as transient receptor potential (TRP) channels, are the essential components involved in SOCE, which plays an important role in increasing [Ca$^{2+}$]$_{cyt}$ and refilling Ca$^{2+}$ into the intracellular stores.

The precise components of ROC and SOC have remained a mystery and may be different among different types of cells. However, recent studies suggest that in vascular smooth muscle and endothelial cells, canonical TRP (TRPC) channels are involved in forming functional ROC and SOC. TRPC channels are members of the mammalian TRP family, which has 28 members that are divided into six structural subfamilies. TRPC channels multimerize to form homo- or heterotetramers that function as voltage-independent nonselective cation channels permeable to Ca$^{2+}$/Na$^+/$K$^+$/Cs$^+$/Li$^+$, and Mg$^+$ (13, 84, 110). TRPC1 is one of the pore-forming subunits of functional SOC in the pulmonary vasculature and is critical for pulmonary vasoconstriction and PASMC proliferation induced by SOCE (9, 18, 62, 79, 80, 116). TRPC1 is unlikely to form homomeric channels but has been shown to heteromultimerize with TRPC3, TRPC4, and TRPC5.
TRPC4, and TRPC5 (9). Interestingly, a novel mechanism of IP3-induced TRPC activation has been reported. In rabbit portal vein smooth muscle cells, IP3 generation can potentiate TRPC6/C7 activity by removing the inhibitory effect of phosphatidylinositol 4,5-bisphosphate independently of IP3R activation (55).

SOCE, or capacitative Ca\(^{2+}\) entry, was originally thought to be mainly a TRPC-dependent mechanism. More recent studies have demonstrated a role for Ca\(^{2+}\) release-activated Ca\(^{2+}\) (CRAC) channels in SOCE with STIM and Orai as major components. STIM and Orai were originally characterized in Drosophila, and shortly thereafter, mammalian homologs STIM1 and -2 and Orai1, -2, and -3 were identified (20, 21, 81, 96, 145). STIM1 is a 685 amino acid-long single-transmembrane protein that is mainly expressed on the SR/ER membrane (and also in plasma membrane) and contains an EF-hand domain near the NH2-terminus that senses the Ca\(^{2+}\) concentration in the SR/ER ([Ca\(^{2+}\)]\(_{\text{SR/ER}}\)). Under normal conditions, [Ca\(^{2+}\)]\(_{\text{SR/ER}}\) is ~1 mM, and after IP3R activation the concentration can be depleted or significantly decreased to ~300 \text{\mu}M. A decrease in the [Ca\(^{2+}\)]\(_{\text{SR/ER}}\) results in less Ca\(^{2+}\) bound to the EF-hand domain of STIM1 (Fig. 8). When Ca\(^{2+}\) is not bound to the EF-domain, STIM1 then undergoes a conformational change, which allows it to multimerize and translocate to the SR/ER-plasma membrane junction (or puncta) where it interacts with Orai1 homo- or heterotetramers on the plasma membrane, activates SOC, and induces SOCE (Fig. 8) (96, 145). Exactly how STIM migrates to the puncta to interact with Orai in the plasma membrane is not known, but as the details emerge, it may provide interesting insights on how STIM anchors to microtubules and on any additional scaffolding features that STIM has to keep the SR/ER intact.

Orai1 is the pore-forming unit for CRAC channels in the plasma membrane (20, 86). STIM1 is capable of binding all three Orai homologs with the interaction first shown by coimmunoprecipitation (130). Many reports show that it is the cytosolic COOH-termini of STIM1 that acts as the effector to Orai and TRPC channels. When this domain is expressed on its own, it is sufficient to constitutively activate CRAC channels independently of Ca\(^{2+}\) store depletion (20). This suggests that STIM operates as an effector with some degree of promiscuity with pore-forming channels on the plasma membrane to mediate Ca\(^{2+}\) influx.

Both ROC and SOC, when activated, allow Ca\(^{2+}\) to flow into the cytosol. Resting [Ca\(^{2+}\)]\(_{\text{cyt}}\) is in the neighborhood of ~100 nM, whereas the extracellular concentration is roughly 10,000-fold higher, near ~2 mM. As a result, when ROC and SOC are open, Ca\(^{2+}\) influx is driven by the electrochemical gradient.
gradient and $[\text{Ca}^{2+}]_{\text{cyt}}$ rises without the expense of energy. Ca$^{2+}$ can be pumped from the cytosol to extracellular or intercellular space against its electrochemical gradient. Extrusion of Ca$^{2+}$ through the plasma membrane is mediated by the plasma membrane Ca$^{2+}$-Mg$^{2+}$ ATPase, or Ca$^{2+}$-pump, and the Na$^+$/Ca$^{2+}$ exchanger (NCX). The action of these Ca$^{2+}$ transporters are required for homeostatic levels of $[\text{Ca}^{2+}]_{\text{cyt}}$ and are vital in maintaining the $\sim 100$ mM $[\text{Ca}^{2+}]_{\text{cyt}}$ (37, 68, 82, 142). The NCX has a larger capacity than the plasma membrane Ca$^{2+}$-Mg$^{2+}$ ATPase and is one of the important mechanisms for Ca$^{2+}$ extrusion when $[\text{Ca}^{2+}]_{\text{cyt}}$ is increased and cytosolic Na$^+$ concentration ($[\text{Na}^+]_{\text{cyt}}$) is low. Mammalian cells maintain a low $[\text{Na}^+]_{\text{cyt}}$ compared with the extracellular concentration of Na$^+$. The transmembrane Na$^+$ gradient can be used to energize the NCX, which moves three Na$^+$ in per one Ca$^{2+}$ out. Nevertheless, a rise in $[\text{Na}^+]_{\text{cyt}}$ (especially in the local submembrane area) converts the NCX from the forward mode (Ca$^{2+}$ out and Na$^+$ in) to the reverse mode (Ca$^{2+}$ in and Na$^+$ out), which becomes an important mechanism to increase $[\text{Ca}^{2+}]_{\text{cyt}}$ (Fig. 7). Studies have shown that the reverse mode of NCX contributes to the increased $[\text{Ca}^{2+}]_{\text{cyt}}$ in pulmonary and systemic vascular smooth muscle cells, which can lead to pulmonary and systemic hypertension (50, 95, 118, 141). The inward transportation of Ca$^{2+}$ through the reverse model of NCX is important for maintaining a high $[\text{Ca}^{2+}]_{\text{cyt}}$ and refilling Ca$^{2+}$ into the SR/ER via SERCA, when $[\text{Na}^+]_{\text{cyt}}$ is increased locally by increased Na$^+$ influx through TRPC channels (64).

The increase in $[\text{Ca}^{2+}]_{\text{cyt}}$ due to SOCE also activates the Ca$^{2+}$-activated Cl$^-$ (ClCa) channels in PASMCs (1, 38, 129). Since intracellular [Cl$^-$] is relative high (up to 50 mM), the equilibrium potential for Cl$^-$ ($E_{\text{Cl}}$) is less negative than the resting $E_m$. So the activation of ClCa channels by SOCE would induce Cl$^-$ efflux or inward currents and cause membrane depolarization, which subsequently opens VDCC and further increases $[\text{Ca}^{2+}]_{\text{cyt}}$ in PASMCs. The functional interaction among SOCE, ClCa channel activation, membrane depolarization, and VDCC activation are actually an important mechanism involved in the development of sustained pulmonary vasoconstriction and vascular medial hypertrophy in animals and patients with pulmonary hypertension (1, 38, 71, 129).

Upregulated Expression and Enhanced Function of TRPC Channels in PASMCs from Patients with IPAH

The elevation of intracellular Ca$^{2+}$ can activate signal transduction pathways important for the stimulation of transcription factors required for cell-cycle progression and proliferation, a key component of vascular remodeling (11, 46). TRPC channel function plays an important role in regulating $[\text{Ca}^{2+}]_{\text{cyt}}$ during this process. In normal PASMCs, proliferation is associated with an increase in both mRNA and protein expression of TRPC1 and TRPC6 (42, 116, 133, 134). Consistent with unregulated TRPC expression, proliferating PASMCs have a significantly greater amplitude of SOCE than that of growth-arrested cells. Inhibition of TRPC expression with antisense oligonucleotides markedly decreases the amplitude of SOCE and significantly inhibits the proliferation of PASMCs (116).

In the PASMCs from patients with IPAH, the resting $[\text{Ca}^{2+}]_{\text{cyt}}$ is higher than in cells from normal subjects and normotensive control patients. Additionally, the amplitude of SOCE, induced by store depletion using cyclopiazonic acid, is significantly greater in PASMCs from patients with IPAH than...
in cells from control patients (132, 133). Along with increased SOCE, the mRNA and protein expression of TRPC3 and TRPC6 are much greater in PASMCs from patients with IPAH than in PASMCs from control patients or those with secondary pulmonary hypertension (42). In addition to upregulated TRPC3/C6 channels, STIM2/Orai3 are upregulated in IPAH-PASMCs compared with normal PASMCs (108). The effect of increased expression levels of TRPC channels (and STIM2/Orai3) translates to enhanced growth and proliferation of IPAH-PASMCs compared with normal PASMCs (Fig. 9).

Furthermore, when TRPC6 protein levels are dampened with small interfering RNA, the proliferative effect is significantly decreased (132). A recent report has identified a unique genetic variation associated with the TRPC6 gene in patients with IPAH. The “gain of function” single-nucleotide polymorphism in the promoter of TRPC6 gene may link the inflammatory mediators present before the onset of IPAH to the upregulation of TRPC6 channels and the augmented \([\text{Ca}^{2+}]_{\text{cyt}}\) and proliferation of PASMCs in patients with IPAH (133). In addition, hypoxic treatment of PASMCs from normal humans subjects causes increase in basal \([\text{Ca}^{2+}]_{\text{cyt}}\) that is SOC dependent and is accompanied by upregulation of TRPC1 (65). Enhanced \([\text{Ca}^{2+}]_{\text{cyt}}\), resulting from SOC activation also leads to nuclear translocation of NFAT, which is involved in hypoxia-induced PASMC proliferation (124). It has been shown that sildenafil, a potent phosphodiesterase type-5 inhibitor, attenuates the hypoxic response and TRPC1 expression and decreases NFAT nuclear translocation, suggesting that sildenafil may interrupt this pathway and provide effective therapy in targeting the progression of pulmonary remodeling in PAH.

Upregulated NCX in PASMCs from Patients with IPAH

There have been a number of studies that have demonstrated that the plasma membrane NCX is implicated in the regulation of \([\text{Ca}^{2+}]_{\text{cyt}}\) homeostasis in vascular smooth muscle cells (64, 66, 117). As mentioned earlier, TRPC channels are permeable to \([\text{Ca}^{2+}]_{\text{cyt}}\) and \([\text{Na}^{+}]_{\text{cyt}}\); the permeability of many TRPC channels to \([\text{Na}^{+}]_{\text{cyt}}\) is greater than to \([\text{Ca}^{2+}]_{\text{cyt}}\). Therefore, when SOC are opened upon store depletion or ROC are opened upon receptor activation, both \([\text{Ca}^{2+}]_{\text{cyt}}\) and \([\text{Na}^{+}]_{\text{cyt}}\) would occur. The \([\text{Na}^{+}]_{\text{cyt}}\) influx through TRPC-formed SOC or ROC would increase \([\text{Na}^{+}]_{\text{cyt}}\) and results in the transition of NCX from the forward mode (\([\text{Ca}^{2+}]_{\text{out}}\) and \([\text{Na}^{+}]_{\text{in}}\)) to the reverse mode (\([\text{Ca}^{2+}]_{\text{in}}\) and \([\text{Na}^{+}]_{\text{out}}\)), leading to the inward transportation of \([\text{Ca}^{2+}]_{\text{cyt}}\) and increase in \([\text{Na}^{+}]_{\text{cyt}}\). The reverse mode of NCX has been shown to couple with TRPC6. Localized increases in \([\text{Na}^{+}]_{\text{cyt}}\), generated by \([\text{Na}^{+}]_{\text{cyt}}\) influx through TRPC6 channels drive the reversal NCX and mediate inward transportation of \([\text{Ca}^{2+}]_{\text{cyt}}\) in smooth muscle cells (64). Additionally, in human bronchial smooth muscle cells, store depletion has been linked to NCX activation via STIM1 and plays an important role in \([\text{Ca}^{2+}]_{\text{cyt}}\) homeostasis (66). We have shown that protein and mRNA expression of NCX is upregulated in PASMCs isolated from patients with IPAH and secondary pulmonary hypertension (142). Removal of the extracellular \([\text{Na}^{+}]\), by decreasing

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**Fig. 9.** The pathogenic role of TRPC6 and NCX in idiopathic PAH (IPAH). A: genetic mutations and/or environmental stimulation leads to upregulation of TRPC6 resulting in an increased number of ROC and SOC channels in the plasma membrane and enhanced ROCE and SOCE. The enhanced ROCE/SOCE leads to not only increased \([\text{Ca}^{2+}]_{\text{cyt}}\), but also locally increased cytosolic \([\text{Na}^{+}]_{\text{cyt}}\) concentration and activation of the reverse mode of NCX. NCX is also upregulated in patients with IPAH. These mechanisms contribute to increased \([\text{Ca}^{2+}]_{\text{cyt}}\) and pulmonary vascular remodeling. B and C: representative currents (I) elicited by a ramp depolarization from −100 to +100 mV and \([\text{Ca}^{2+}]_{\text{cyt}}\) in normal or control PASMCs and IPAH-PASMCs before and after treatment with 1-oleoyl-2-acetyl-sn-glycerol (OAG), a membrane permeable DAG analog. B and C reproduced from Yu et al. (133) with permission.
transmembrane Na$^+$ gradient, activates the reverse mode of NCX, resulting in a rapid increase in [Ca$^{2+}$]$_{cyt}$, which is significantly enhanced in IPAH-PASMCs (142, 144). When compared with controls, the increased expression of NCX leads to enhanced SOCE by the reverse mode but did not concomitantly accelerate Ca$^{2+}$ efflux in the forward mode (144). These data indicate that upregulated NCX and enhanced SOCE due to the reverse mode of NCX are additional mechanisms responsible for the increased [Ca$^{2+}$]$_{cyt}$ in PASMCs from patients with IPAH. Hence, NCX modulation may pose an interesting therapeutic target in treating PAH.

Upregulated Caveolin and Increased Number of Caveolae in PASMCs from Patients with IPAH

Caveolae are cholesterol- and sphingosine-rich invaginations in the plasma membrane, of which the main principal structural component is caveolin. They are found in various cell types and facilitate endocytosis, transcytosis, and Ca$^{2+}$ mobilization in addition to acting as scaffold proteins to orchestrate signaling events (41, 94). There are three isoforms of caveolin: Cav1, Cav2, and Cav3 (Fig. 10). Cav2 is localized in the Golgi apparatus but can translocate to the plasma membrane. Cav3 is muscle specific and is more closely related to Cav1 in terms of amino acid sequence (101). Caveolae and caveolin have been implicated in both the human and mouse diseased states of PAH but with some contrasting features. Although there is a decrease in Cav1 expression in whole lung lysates from the pulmonary arteries of IPAH versus control patients, immunohistochemical studies on formalin-fixed lung sections show a substantial increase in Cav1 expression in the smooth muscle layer but not the endothelial layer of pulmonary arterioles from patients with IPAH. In agreement with these data, Western blot analysis demonstrates increased protein expression of both Cav1 and Cav2 in PASMCs from patients with IPAH compared with normal PASMCs (Fig. 10A). In addition, electron microscopy shows that IPAH-PASMCs express more Cav1 and form more caveolae than in normal PASMCs (Fig. 10) (85). An increase in caveolae contributes to a marked rise in SOCE and DNA synthesis. Downregulation of caveolin with Cav1 small interfering RNA or disruption of caveolae with disrupting agents (e.g., MβCD) attenuates SOCE and decreases DNA synthesis in IPAH-PASMCs (85, 100). Also, overexpressing Cav1 in normal PASMCs has the opposite effect and increases DNA synthesis and SOCE. However, these results contrast somewhat with the animal models of pulmonary hypertension. Cav1$^{-/-}$ mice show pathological features similar to a pulmonary hypertension phenotype such as right ventricle hypertrophy, increased medial thickness, and muscularization of distal pulmonary vessels (146, 147). In agreement with Cav1-deficient mice, rat models of pulmonary hypertension induced by monocrotaline or hypoxia exhibit a decrease in Cav1 expression, albeit mainly in endothelial cell isoform Cav1$\alpha$. The reduced expression is noted as early as 48 h after monocrotaline challenge, but importantly the attenuated expression is predominantly found in the intimal layer (endothelium) of the pulmonary arteries (69). The above results highlight that caveolae and caveolin are critical regulators in the pathogenic mechanisms of pulmonary hypertension with...
some localization- and species-specific effects in humans versus rodents.

Functional Coupling of ROC/SOC and NCX in Caveolae Is an Important Mechanism for Sustained Pulmonary Vasoconstriction and Medial Hypertrophy in Patients with IPAH

Caveolae are important for colocalizing receptors (e.g., GPCR and RTK) into microdomains and aid in an efficient activation of signal transduction pathways. Ligands accumulate in the caveolae and activate these receptors, leading to increased production of DAG and IP\(_3\). DAG activates ROC, and IP\(_3\) activates the IP\(_3\)R on the SR/ER, releasing Ca\(^{2+}\) into the cytosol and depleting the store. Store depletion activates TRPC-formed SOC and/or Orail-formed SOC via STIM translocation and causes SOCE. Cav1 facilitates the localization of TRPC1 (and other TRPC isoforms) into caveolae through interaction with the caveolin-scaffolding domain found in the COOH-terminal of TRPC1 (Fig. 10B) (18, 63, 113). The activation of TRPC channels promotes Ca\(^{2+}\) and Na\(^{+}\) influx. The local accumulation of Na\(^{+}\) reverses the outward transport of Ca\(^{2+}\) via NCX, which is also localized to caveolae (97), and further enhances Ca\(^{2+}\) entry. The close vicinity of the receptors and channels in the caveolae with the SR ensures efficient agonist-induced increase in [Ca\(^{2+}\)]\(_{cyt}\), vasoconstriction, and pulmonary vascular wall thickening (Fig. 10B) (100). All of the components of this scenario (TRPC, Cav-1, caveolae, and NCX) are upregulated in PASMCs from patients with IPAH (85). These observations demonstrate that a functional coupling of GPCR, RTK, ROC/SOC, TRPC, and NCX in caveolae contributes to increased [Ca\(^{2+}\)]\(_{cyt}\), resulting in increased proliferation of PASMCs and vasoconstriction in patients with IPAH.

Downregulated K\(_v\) Channels, Membrane Depolarization and Opening of VDCC Contribute to Increases in [Ca\(^{2+}\)]\(_{cyt}\) in PASMCs from Patients with IPAH

Downregulated and dysfunctional K\(_v\) channels have also been shown to impact Ca\(^{2+}\) signaling in patients with PAH. The amplitude of whole cell K\(_v\) currents and the mRNA/protein expression of K\(_v\) channels are significantly decreased in PASMCs from patients with IPAH compared with normotensive patients (135, 140). Additionally, PASMCs isolated from patients with IPAH have a depolarized resting membrane potential and a higher resting [Ca\(^{2+}\)]\(_{cyt}\) and display a blunted response to K\(_v\) channel blockers (135). Decreased expression or compromised function of K\(_v\) channels causes membrane depolarization, which activates VDCC, enhances voltage-dependent Ca\(^{2+}\) entry, and leads to PASMC contraction, migration, and proliferation (Fig. 11).

In addition, changes in K\(_v\) channel activity and expression have been implicated in acute hypoxia-mediated pulmonary vasoconstriction. Acute hypoxia inhibits K\(_v\) channels (e.g., K\(_v\)1.5, K\(_v\)1.5/K\(_v\)1.2, KCNQ, K\(_v\)2.1, and K\(_v\)3.1b), causing membrane depolarization and subsequent opening of L-type VDCCs (44, 53, 54, 90, 138, 139). The ensuing elevation of [Ca\(^{2+}\)]\(_{cyt}\) elicits PASMC contraction and pulmonary vasoconstriction. Chronic hypoxia also downregulates K\(_v\) channels (125) and decreases whole cell K\(_v\) currents in PASMCs (89, 105). Loss of function and reduced expression of K\(_v\)1.5 and
Kv2.1 cause sustained increase in \([\text{Ca}^{2+}]_{\text{cyt}}\) and contribute to the progression of pulmonary hypertension through sustained membrane depolarization, cell proliferation, and inhibition of apoptosis (2, 3, 125). Importantly, restoring the expression of Kv1.5 through adenovirus in a chronic hypoxic pulmonary hypertension rodent model reduces the experimental pulmonary hypertension (91).

**Downregulated Kv Channels and Inhibited Apoptosis also Contribute to Pulmonary Vascular Medial Hypertrophy in PAH**

Another consequence of dysfunctional Kv channels is a decrease in apoptosis. One of the earliest morphological changes seen in cells undergoing apoptosis is apoptotic volume decrease (16). In the early stages of apoptotic volume decrease, K+ and Cl− exit the cell along their electrochemical gradient through an increased number of open K+ and Cl− channels (Fig. 12). Water then leaves the cell through aquaporins in the plasma membrane to maintain the osmotic balance, thus causing cell shrinkage. Inhibition of Kv channels in PASMCs from patients with IPAH and animals with chronic hypoxia-induced pulmonary hypertension inhibits apoptotic volume decrease and attenuates apoptosis (143).

Cytoplasmic K+ is an inhibitor of caspases and nucleases, and the maintenance of sufficient K+ in the cytosol due to decreased activity of K+ channels can inhibit apoptosis by attenuating the activity of intracellular caspases (19, 48, 58–60). In PASMCs from patients with IPAH, apoptosis induced by bone morphogenetic protein or staurosporine is inhibited compared with normal PASMCs (143). Additionally, an overexpression of Kv1.5 in normal PASMCs accelerates staurosporine-mediated cell shrinkage and enhances apoptosis (19). These data suggest that downregulated Kv channels in PASMCs from patients with IPAH contribute to decreased apoptosis as well as pulmonary vasoconstriction and increased PASMC proliferation because of increased \([\text{Ca}^{2+}]\) (Fig. 12).

**Summary and Future Directions**

PAH is a fatal disease that predominantly affects women. Sustained pulmonary vasoconstriction (due to PASMCs contraction) and excessive pulmonary vascular remodeling (due partially to PASMC migration and proliferation) are the major causes for the elevated PVR in patients with PAH. Increased PVR demands the right heart to work harder and can lead to right ventricular hypertrophy, right heart failure, and death. A reoccurring theme in the pathogenic mechanism of PAH is the involvement of \(\text{Ca}^{2+}\) signaling. Increased \([\text{Ca}^{2+}]_{\text{cyt}}\) in PASMCs can stimulate vasoconstriction and vascular remodeling. A rise in \([\text{Ca}^{2+}]_{\text{cyt}}\) due to the enhanced SOCE/ROCE and voltage-dependent \(\text{Ca}^{2+}\) entry in IPAH-PASMCs also activates many signal transduction proteins (e.g., CaMK, PKC, MAPK, and calcineurin) and transcription factors (e.g., activator protein-1, NFAT, CREB, and NF-κB), thereby affecting gene expression and promoting cell proliferation (Fig. 13). \(\text{Ca}^{2+}\) signaling activates cross talk between multiple genetic pathways and involves an interaction network of numerous genes and proteins. Several factors, such as increased expres-

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**Fig. 13.** The positive-feedback loop hypothesis of \(\text{Ca}^{2+}\) signaling in PAH. Multiple \(\text{Ca}^{2+}\) channels (ROC, SOC, VDCC, TRPC) contribute to regulating the \([\text{Ca}^{2+}]_{\text{cyt}}\) in PASMC (A). An increase in \([\text{Ca}^{2+}]_{\text{cyt}}\), because of increased expression and/or function of \(\text{Ca}^{2+}\) channels (or transporters), can lead to the activation of transcription factors and signaling pathways in a positive-feedback loop, which leads to a greater and persistent influx of \(\text{Ca}^{2+}\). The activated signaling pathways result in cross talk among many different genetic and signaling pathways and an interaction network of multiple genes and proteins (B). This ultimately leads to the processes (contraction, migration, imbalanced ratio of proliferation to apoptosis, misguided differentiation, dedifferentiation, transdifferentiation, partial reprogramming) in all cell types involved in the pulmonary vascular wall, which can contribute to the concentric pulmonary vascular remodeling (C) and, ultimately, PAH.
sion of TRPC, Orai, STIM, and NCX, as well as the enhanced functional coupling of TRPC and NCX in an increased number of caveolae in PASMCs, have been identified in patients with IPAH. Furthermore, downregulated and/or dysfunctional Kv channels have been implicated in PASMCs from patients with IPAH, which contribute to increasing [Ca\(^{2+}\)]\(_{\text{cyt}}\) by causing membrane depolarization and promoting voltage-dependent Ca\(^{2+}\) entry (Fig. 13). All these observations point to altered Ca\(^{2+}\) signaling as one of the major pathogenic mechanisms of PAH.

The proteins or subunits involved in forming functional SOC/ROC (e.g., TRPC and/or STIM/Orai) and VDCC (e.g., CACNA1C/Cav1.2), as well as the Kv channel α-subunits (e.g., Kv1.5), are oppositely regulated by the Ca\(^{2+}\)-dependent/sensitive transcription factors (Fig. 13). The resulting upregulation of SOC/ROC and VDCC and downregulation of Kv channels would enhance the Ca\(^{2+}\) influx and, ultimately, increase [Ca\(^{2+}\)]\(_{\text{cyt}}\) in PASMCs. The positive-feedback loop in the regulation of [Ca\(^{2+}\)]\(_{\text{cyt}}\), shown in Fig. 3A, may be an important cellular (or pathogenic) mechanism responsible for the enhanced Ca\(^{2+}\) signaling (or increased [Ca\(^{2+}\)]\(_{\text{cyt}}\)) in PASMCs from patients and animals with PAH.

Increased activity or upregulation of the proteins involved in forming SOC/ROC and VDCC suggests that these channels may in fact be potentially valuable targets for developing novel therapeutic approaches using RNAi delivery or nanoparticle-driven inhibitors that antagonize functional coupling of these proteins. Additionally, restoring the function of Kv channels could provide a reversal of apoptotic resistant PASMCs and ameliorate the medial hypertrophy associated with PAH (15, 72). Restoring protein function can be a challenging task in a diseased state, but as more is known regarding microRNA regulation of the players in PAH, potential targets may emerge (27).

As research continues, we hope to discover connections between Ca\(^{2+}\) signaling and other pathways to map out an interaction network to further our understanding of the pathogenic mechanisms of PAH with the goal of improving the prognosis for patients with PAH. Furthermore, it is important to reveal the molecular and cellular mechanisms involved in the upregulation of SOC/ROC (and the receptors that are functionally coupled to the ROC/SOC) and downregulation of Kv channels and to identify the transcription factors responsible for the up- and downregulation of these proteins in PASMCs from patients and animals with PAH. Eventually, the goal is to identify a therapeutic regimen, or a therapeutic “cocktail,” targeting multiple channels in the Ca\(^{2+}\) signaling pathways, which is more effective and specific (than currently used VDCC blockers, such as nifedipine) for PAH.

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AUTHOR CONTRIBUTIONS
F.K.K. and J.X.-J.Y. prepared figures; F.K.K., M.Y.S., and J.X.-J.Y. drafted manuscript; F.K.K., K.A.S., and J.X.-J.Y. edited and revised manuscript; J.X.-J.Y. conceived and designed research; J.X.-J.Y. analyzed data; J.X.-J.Y. interpreted results of experiments.

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