HIGHLIGHTED TOPIC | Pulmonary Circulation and Hypoxia

Hypoxic pulmonary vasoconstriction: role of ion channels

Joseph R. H. Mauban, Carmelle V. Remillard, and Jason X.-J. Yuan
Department of Medicine, University of California, San Diego, La Jolla, California

Mauban, Joseph R. H., Carmelle V. Remillard, and Jason X.-J. Yuan. Hypoxic pulmonary vasoconstriction: role of ion channels. J Appl Physiol 98: 415–420, 2005. doi:10.1152/japplphysiol.00732.2004.—Acute hypoxia induces pulmonary vasoconstriction and chronic hypoxia causes structural changes of the pulmonary vasculature including arterial medial hypertrophy. Electro- and pharmacomechanical mechanisms are involved in regulating pulmonary vasomotor tone, whereas intracellular Ca\textsuperscript{2+} serves as an important signal in regulating contraction and proliferation of pulmonary artery smooth muscle cells. Herein, we provide a basic overview of the cellular mechanisms involved in the development of hypoxic pulmonary vasoconstriction. Our discussion focuses on the roles of ion channels permeable to K\textsuperscript{+} and Ca\textsuperscript{2+}, membrane potential, and cytoplasmic Ca\textsuperscript{2+} in the development of acute hypoxic pulmonary vasoconstriction and chronic hypoxia-mediated pulmonary vascular remodeling.

Hypoxia; proliferation; remodeling; calcium

Vasoconstriction in response to alveolar hypoxia is a unique physiological response observed in pulmonary arteries and arterioles. The acute hypoxia-induced pulmonary vasoconstriction (HPV) is an important mechanism that aids in matching ventilation with perfusion by directing blood flow from poorly ventilated regions of the lung to areas with normal or relatively high ventilation. Although acute HPV benefits gas exchange and maximizes oxygenation of venous blood in the pulmonary artery, sustained HPV or chronic exposure to hypoxia is a major cause for the elevated pulmonary vascular resistance (PVR) and pulmonary arterial pressure (PAP) in patients with pulmonary arterial hypertension (PAH) associated with hypoxic cardiopulmonary diseases.

On the basis of the Poiseuille principle, PVR is inversely proportional to the fourth power of the radius of the pulmonary artery. Therefore, small changes in the radius of pulmonary arteries (especially small arteries and arterioles) can significantly change PVR. Pulmonary vasoconstriction, vascular remodeling (characterized with medial and intimal hypertrophy), and in situ thrombosis are three major factors that can decrease the radius of pulmonary vessels and increase PVR and, ultimately, PAP.

Experiments in vitro demonstrate that acute hypoxia causes constriction in isolated pulmonary arteries with or without intact endothelium and induces contraction in isolated pulmonary artery smooth muscle cells (PASMC) via changes in membrane potential ($E_m$), cytosolic free Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{cyt}), and Ca\textsuperscript{2+} sensitivity of contractile apparatus, and myosin light chain phosphorylation (13, 17, 22). These observations indicate that both sensor and effector mechanisms essential for acute HPV reside in PASMC. However, some studies have suggested that not all components necessary for the development of HPV are present in PASMC but may include input from endothelial cells and fibroblasts (45).

ROLE OF [Ca\textsuperscript{2+}]\textsubscript{cyt} IN PASMC CONTRACTION AND PROLIFERATION

An increase in [Ca\textsuperscript{2+}]\textsubscript{cyt} in PASMC is a major trigger for pulmonary vasoconstriction. Acute hypoxia causes a slow graded depolarization and increase in [Ca\textsuperscript{2+}]\textsubscript{cyt}. Both the depolarization and Ca\textsuperscript{2+} increase are directly proportional to the severity of hypoxia. The biphasic rise in [Ca\textsuperscript{2+}]\textsubscript{cyt} is generally agreed to have two components: an initial Ca\textsuperscript{2+} release from intracellular stores and concurrent Ca\textsuperscript{2+} influx from the extracellular compartment via voltage-dependent and -independent mechanisms (14, 18). A rise in [Ca\textsuperscript{2+}]\textsubscript{cyt} also serves as an important stimulus for cell migration, proliferation, and gene expression (14, 18). As shown in Fig. 1, an increase in [Ca\textsuperscript{2+}]\textsubscript{cyt} not only causes PASMC contraction by activating myosin light chain kinase but also promotes PASMC proliferation by stimulating quiescent cells to enter the cell cycle and 2) by driving proliferating cells through the cell cycle and mitosis. Intracellular Ca\textsuperscript{2+} also activates cytoplasmic signal transduction proteins that are directly or indirectly involved in promoting cell proliferation (Fig. 1). Therefore, central to the understanding of the development of HPV is a working knowledge of the processes that control [Ca\textsuperscript{2+}]\textsubscript{cyt} in PASMC and how hypoxia affects them.

Regulation of [Ca\textsuperscript{2+}]\textsubscript{cyt} in PASMC is achieved mainly in two different ways: 1) Ca\textsuperscript{2+} influx through Ca\textsuperscript{2+} channels or Ca\textsuperscript{2+} extrusion through the Ca\textsuperscript{2+}-Mg\textsuperscript{2+} ATPase located in the plasma membrane, and 2) Ca\textsuperscript{2+} mobilization (or release) from the sarcoplasmic reticulum (SR) through the inositol-1,4,5-trisphosphate (IP\textsubscript{3}) and ryanodine receptor (RyR) or Ca\textsuperscript{2+} sequestration by the Ca\textsuperscript{2+}-Mg\textsuperscript{2+} ATPase in the SR membrane (4).
Ca$^{2+}$ influx through the plasma membrane involves multiple Ca$^{2+}$-permeable channels including 1) voltage-dependent Ca$^{2+}$ channels (VDCC), which are regulated by changes in $E_{m}$; 2) receptor-operated Ca$^{2+}$ channels (ROC), which are activated by interaction of agonists with respective receptors and the downstream signaling proteins (e.g., diacylglycerol, IP$_3$, activated by interaction of agonists with respective receptors and second messenger)$^{2}$ receptor-operated Ca$^{2+}$ (SOC), which are opened by depletion of Ca$^{2+}$ from the SR (23, 37). The excitation-contraction coupling processes in pulmonary vascular smooth muscle depend on the function of all these channels. A change in $E_{m}$, for example, is required for the electromechanical coupling that alters vascular tone by regulating the activity of VDCC, which are opened by membrane depolarization and closed by membrane hyperpolarization (23, 28). Interactions of agonists (e.g., norepinephrine, phenylephrine, serotonin, endothelin-1) with membrane receptors are required for the pharmacomechanical coupling that alters vascular tone by regulating the activity of ROC and SOC.

**ROLE OF K$^+$ CHANNELS IN HPV**

As mentioned earlier, $E_{m}$ regulates pulmonary vascular tone by controlling Ca$^{2+}$ influx through VDCC in PASMC. The resting $E_{m}$ is primarily determined by K$^+$ permeability and K$^+$ concentration gradient across the plasma membrane; therefore, the activity of K$^+$ channels in the plasma membrane is a critical determinant of $E_{m}$. Inhibition of K$^+$ channels causes membrane depolarization, opens VDCC, promotes Ca$^{2+}$ influx, increases [Ca$^{2+}$]$_{cyt}$, and triggers PASMC contraction.

In PASMC, there are at least five functionally-distinguishable K$^+$ channels: 1) voltage-gated K$^+$ (Kv) channels, 2) Ca$^{2+}$-activated K$^+$ (KCa) channels, 3) ATP-sensitive K$^+$ (K_ATP) channels, 4) inward rectifier K$^+$ (Kir) channels, and 5) tandem pore domain K$^+$ (K_T) channels. Although all K$^+$ channels contribute to regulating $E_{m}$, Kv and K_T channels have been demonstrated to be the major K$^+$ channels regulating the resting $E_{m}$ in PASMC (24).

Blockade of Kv channels in PASMC with 4-aminopyridine (4-AP) causes membrane depolarization, induces Ca$^{2+}$-dependent action potentials, increases [Ca$^{2+}$]$_{cyt}$, and causes cell contraction (34, 56). Acute hypoxia ($<$3 min) significantly reduces Kv channel activity or whole-cell Kv currents $[I_{K(N)}]$, and causes membrane depolarization in PASMC, but not in smooth muscle cells from systemic arteries (e.g., mesenteric and renal arteries) (57). These observations suggest that hypoxia-induced depolarization and Ca$^{2+}$-dependent action potentials in pulmonary arteries (13) occur, at least partially, as a result of functional inhibition of Kv channels and decreased $I_{K(N)}$ (26, 34, 35, 57). The early steps of HPV therefore appear to involve inhibition of $I_{K(N)}$, $E_{m}$ depolarization, and increased [Ca$^{2+}$]$_{cyt}$ due to Ca$^{2+}$ influx via VDCC, which ultimately leads to pulmonary vasoconstriction.

Native Kv channels are homo- or heterotetramers composed of the pore forming α-subunits and the cytoplasmic regulatory β-subunits. Multiple Kv channel gene products, including homotetrameric channels of Kv1.2, Kv1.3, Kv2.1, and Kv3.1 subunits or heterotetrameric channels of Kv1.2/Kv1.5, Kv1.5/ Kv3.1, and Kv3.1/Kv9.3 subunits have demonstrated sensitivity to acute hypoxia (5). Among these, the Kv1.5 α-subunit has been identified as a putative component of native Kv channels in hypoxia-sensitive PASMC. A study using Kv1.5 knockout mice showed that the Kv1.5 α-subunit has an important role in HPV and in the hypoxia-induced reduction of $I_{K(N)}$ and membrane depolarization (3). A more recent study also demonstrated that in vitro transfer of the Kv1.5 gene inhibited pulmonary arterial hypertension and restored HPV in chronically hypoxic rats (36). Although acute hypoxia reduces $I_{K(N)}$ by inhibiting Kv channel function, chronic exposure to hypoxia causes sustained reduction of $I_{K(N)}$ by downregulating Kv channel gene expression (19, 31, 36, 43, 44, 47).

How acute hypoxia inhibits Kv channel function and how chronic hypoxia downregulates Kv channel gene expression remain unclear. The potential intermediates or mechanisms involved in hypoxia-mediated inhibition of Kv channel function and expression include oxygen radicals, mitochondrial electron chain transport, NADPH oxidase, redox status, metabolic inhibition, cytochrome P-450 oxidoreductase, cytochrome c, cellular redox status changes, endothelin-1 production, and conformational changes of α- and β-subunits (e.g., due to cysteine reduction of the channel protein), and phosphorylation of α- and β-subunits (30).

In addition to Kv channels, acute hypoxia also inhibits K_T (12, 27) and K_Ca (16, 29) channels in PASMC. The resultant membrane depolarization or inhibition of membrane repolarization would further enhance the activity of VDCC and increase [Ca$^{2+}$]$_{cyt}$ and ultimately would sustain the acute HPV.
A direct augmenting effect of acute hypoxia on VDCC has also been demonstrated in PASMC from small pulmonary arteries (10). In summary, one of the important mechanisms of acute HPV seems to be the inhibition of Kv channels (and other types of K^+ channels to a lesser extent), which causes membrane depolarization, promotes Ca^{2+} influx via VDCC, increases [Ca^{2+}]_cyt, and triggers membrane potential (E_m) depolarization, 2) directly activating voltage-dependent Ca^{2+} channels (VDCC), 3) activating inositol-1,4,5-triphosphate receptor (IP_3-R) and ryanodine receptors (RyR) in the sarcoplasmic reticulum (SR) and inducing Ca^{2+} release, 4) indirectly activating store-operated Ca^{2+} channels (SOC), and the resultant decrease in Kv currents causes E_m depolarization, opens VDCC, and increases [Ca^{2+}]_cyt, whereas the increase in SOC activity enhances voltage-independent CCE and raises [Ca^{2+}]_cyt, ultimately causing sustained pulmonary vasoconstriction and stimulating PASMC proliferation. Chronic hypoxia also upregulates TRPC channel expression, enhances CCE, increases [Ca^{2+}]_cyt, and enhances AP-1 transcription factor binding activity in pulmonary artery endothelial cells (PAEC). The subsequent expression and release of vasoconstrictors [e.g., endothelin-1 (ET-1)] and growth factors (e.g., PDGF) cause PASMC contraction and proliferation via paracrine mechanisms. DAG, diacylglycerol; GPCR, G protein-coupled receptor; RTK, receptor tyrosine kinase; AP-1, activating protein 1 transcription factors; K_{Ca}, Ca^{2+}-activated K^+ channels; ROC, receptor-operated Ca^{2+} channels.
(2, 20, 52). However, the manner in which these different attributes may contribute, if any, to the long-term responses of arteries (remodeling, proliferation, apoptosis, etc.) remains unresolved.

In addition to regional diversity, it is also becoming apparent that there is a difference in the hypoxic response due to \( K^+ \) channels as PASMC mature from fetal to neonatal and adult PASMC (52). In fetal PASMC, resting \( E_m \) is inhibited by charybdotoxin (but not by 4-AP), \( I_K \) is inhibited more by \( Ca^{2+} \) chelation than by 4-AP, and acute hypoxia and NO can increase \( I_K \) (6, 38). This suggests that \( K_{Ca} \) channel activity, primarily in the form of spontaneous transient outward currents (STOCs), is prominent in hypoxia-induced fetal pulmonary vasodilation. However, in adult PASMC, \( E_m \) is modulated by and acute hypoxia partially inhibits 4-AP-sensitive \( I_K(V) \) to cause pulmonary vasoconstriction (2, 38, 57). This suggests a maturational shift in ion channel expression, which could account for differential responses to acute hypoxia as well as to vasodilators such as NO.

### ROLE OF SR \( Ca^{2+} \) RELEASE IN HPV

In addition to mediating \( Ca^{2+} \) influx in PASMC, acute hypoxia causes \( Ca^{2+} \) release by activating \( IP_3 \) receptors (\( IP_3-R \) and RyR, also referred to as \( Ca^{2+} \) channels and membrane hyperpolarization, it remains unclear whether acute hypoxia is \( Ca^{2+} \) influx inadequate to maintain PASMC contraction. In other words, \( Ca^{2+} \) entry is modulated by and acute hypoxia partially inhibits 4-AP-sensitive \( I_K(V) \) to cause pulmonary vasoconstriction in fetal (2, 38, 57). This suggests a maturational shift in ion channel expression, which could account for differential responses to acute hypoxia as well as to vasodilators such as NO.

**ROLE OF STORE-OPERATED \( Ca^{2+} \) INFLUX IN ACUTE HPV**

Acute hypoxia-induced \( Ca^{2+} \) mobilization causes \( Ca^{2+} \) influx (Fig. 2): 1) the local rise in \( [Ca^{2+}]_{cyt} \), due to \( Ca^{2+} \) release, may open \( Ca^{2+} \)-activated \( Cl^- \) (\( Cl_{Ca} \) ) channels, causing \( E_m \) depolarization, and subsequently activating \( VDCC \) (40, 50, 55); and 2) depletion or emptying of \( Ca^{2+} \) from the SR triggers activator \( Ca^{2+} \) entry (CCE) through SOC (25, 41, 48). Furthermore, the hypoxia-induced \( Ca^{2+} \) release has been suggested to serve as an initial inhibitor for \( K_v \) channels (34), although hypoxia inhibits \( K_v \) channels in the absence of extracellular and intracellular \( Ca^{2+} \) (57). It is likely that acute hypoxia-mediated inhibition of \( K_v \) channels and membrane depolarization result from a \( Ca^{2+} \)-independent mechanism (57). Because \( Ca^{2+} \) release due to activation of RyR and \( IP_3-R \) in the SR is linked to the activation of large-conductance \( K_{Ca} \) channels and induction of membrane hyperpolarization, it remains unclear whether acute hypoxia-mediated \( Ca^{2+} \) release is an initial cause for \( E_m \) depolarization in PASMC. It is thus important to investigate 1) whether hypoxia-sensitive SR pools are closely colocalized with \( Cl_{Ca} \) channels and/or \( K_v \) channels (e.g., in caveolae), but are functionally “dissociated” from \( K_{Ca} \) channels in PASMC; and 2) whether \( K_{Ca} \) channels in PASMC are less sensitive to hypoxia-induced \( Ca^{2+} \) release as a result of hypoxia-mediated inhibition of these \( K_{Ca} \) channels (16, 29).

As for the \( K_v \) channels, it appears that developmental regulation of ryanodine-sensitive stores may also impact the pulmonary response to HPV. Case in point, 50 \( \mu M \) ryanodine caused a substantial \( [Ca^{2+}]_{cyt} \) transient in fetal, but not neonatal or juvenile, rabbit distal PASMC (32), presumably because of enhanced \( Ca^{2+} \) spark activity, that could be blocked by removal of extracellular \( Ca^{2+} \) or by iberiotoxin treatment. In the latter study, ryanodine also evoked charybdotoxin-sensitive STOCs, suggesting a link between SR \( Ca^{2+} \) release channels and \( K_{Ca} \) activity that may modulate pulmonary vascular tone. The enhanced \( Ca^{2+} \) spark and STOC activity in fetal PASMC would therefore favor membrane hyperpolarization in these cells, leading to hypoxia-induced pulmonary vasodilation in the fetus (33).

**ROLE OF STORE-OPERATED \( Ca^{2+} \) INFLUX IN ACUTE HPV AND CHRONIC HYPOXIA-INDUCED PAH**

A nifedipine- and voltage-insensitive, but SK&F 96365- and \( La^{3+} \)-sensitive, \( Ca^{2+} \) entry pathway is active during hypoxia (41, 42). Activation of this pathway seems to be related to hypoxia-induced \( Ca^{2+} \) mobilization from \( IP_3 \) and/or ryanodine-sensitive SR pools. Although \( Ca^{2+} \)-induced \( Ca^{2+} \) influx through sarcolemmal \( Ca^{2+} \) channels may be involved, the store depletion-mediated \( Ca^{2+} \) entry (i.e., CCE) via voltage-independent SOC has been implicated as an additional contributor to the rise in \( [Ca^{2+}]_{cyt} \) and pulmonary vasoconstriction in response to hypoxia (41).

Current lines of thinking state that functional SOC are formed by transient receptor potential (TRP) cation channels (28). Although some TRP channel isoforms are highly expressed in animal and human PASMC (11, 48), the nature of the TRP proteins involved in forming functional SOC and the precise contribution of specific TRP channels to acute HPV and chronic hypoxia-mediated PAH are still incompletely understood. Recent studies in rat PASMC have shown that the acute hypoxia-mediated \( Ca^{2+} \) release was associated with a rise in \( [Ca^{2+}]_{cyt} \) due to CCE; the hypoxia-mediated CCE was sensitive to SK&F 96365, \( Ni^{2+} \), and \( La^{3+} \) (49). This study provides compelling evidence that acute hypoxia-mediated \( Ca^{2+} \) release and store depletion serve as triggers to activate SOC and induce CCE. Heterogeneity of HPV (7) as well as heterogeneity of PASMC in response to acute and chronic hypoxia (45, 52) has been well demonstrated in humans and animals. It is thus possible that different PASMC (e.g., in different branches of the pulmonary arterial tree) may use different mechanisms to induce (and maintain) the rise in \( [Ca^{2+}]_{cyt} \).

In addition to the contribution to acute HPV, CCE and SOC TRP channels in PASMC and pulmonary artery endothelial cells (PAEC) have also been demonstrated to contribute to chronic hypoxia-mediated pulmonary vascular remodeling and sustained pulmonary vasoconstriction. Under normoxic conditions, proliferating PASMC exhibit increased CCE and upregulated TRP channel expression relative to growth-arrested PASMC, suggesting that enhanced SOC activity may be important in mediating pulmonary vascular medial hypertrophy (11, 46, 53). Chronic exposure to hypoxia significantly upregulated canonical TRP (TRPC) channels in PASMC (49) and PAEC (9). In addition, enhanced SOC activity and CCE contribute to the increased tension observed in chronically hypoxic PASMC (15, 49). The hypoxia-mediated TRPC up-regulation in PASMC may contribute to PASMC contraction and proliferation by enhancing receptor- or store-operated...
Ca$^{2+}$ entry and elevating cytoplasmic and nuclear [Ca$^{2+}$]. The hypoxia-mediated TRPC (e.g., TRPC4) upregulation in PAEC enhances amplitude of CCE and SOC currents and increases activating protein 1 transcription factor binding activity (9). Because AP-1 binding sites are present in the promotor region of many genes that are involved in stimulating PASMC proliferation, hypoxia-mediated enhancement of AP-1 binding activity would potentially stimulate synthesis of pulmonary vascular endothelium-derived vasoconstrictors and mitogens, such as PDGF, endothelin-1, and VEGF. These factors may then stimulate PASMC contraction and proliferation via paracrine binding to their receptors in PASMC (Fig. 2).

SUMMARY AND FUTURE RESEARCH

There is now good evidence to indicate that acute HPV and chronic hypoxia-induced PAH are multifactorial processes involving 1) various ion channels and 2) interactions between PASMC and PAEC. Smooth muscle cells contain sensors and effectors that respond to hypoxia. Although they were not discussed in the context of this review, endothelial cells also play a critical role in the development of sustained HPV and vascular structural changes via release of vasoconstrictors and mitogens that may increase [Ca$^{2+}$]$_{cyt}$, increase Ca$^{2+}$ sensitivity of smooth muscle myofilaments, and stimulate PASMC proliferation and migration. Although oxygen-sensing mechanism in PASMC is still controversial, it is generally accepted that hypoxia causes pulmonary vasoconstriction and vascular remodeling. A detailed discussion of the cellular and molecular mechanisms of acute HPV and chronic hypoxia-induced PAH is beyond the scope of this brief review. Readers may wish to refer to accompanying articles in this Highlighted Topics series and a recently published book, titled Hypoxic Pulmonary Vasoconstriction: Cellular and Molecular Mechanisms (54), for a more thorough overview of the topic. It is time for all investigators to work together to figure out a potential “road map” for the mechanisms by which hypoxia causes pulmonary vasoconstriction and vascular remodeling. We believe that the map will be complex and will include multiple cell types, different vasoconstrictors and growth factors, various membrane ion channels and receptors, diverse intercellular and intracellular signal transduction proteins, and a variety of transcription factors. HPV is a critical physiological mechanism to ensure gas exchange and maximal oxygenation of blood. Redundant pathways and multiple mechanisms are likely involved in this physiological response. Persistent HPV or pulmonary vascular remodeling during chronic hypoxia causes pulmonary hypertension that may lead to right heart failure and death, such as in patients with Eisenmenger syndrome and chronic obstructive pulmonary disease. Development of therapeutic approaches targeting hypoxia-sensitive ion channels would benefit or facilitate the treatment of patients with hypoxia-associated pulmonary arterial hypertension.

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