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ROS-Dependent Signaling Mechanisms for Hypoxic Ca^{2+} Responses in Pulmonary Artery Myocytes

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Abstract

Hypoxic exposure causes pulmonary vasoconstriction, which serves as a critical physiologic process that ensures regional alveolar ventilation and pulmonary perfusion in the lungs, but may become an essential pathologic factor leading to pulmonary hypertension. Although the molecular mechanisms underlying hypoxic pulmonary vasoconstriction and associated pulmonary hypertension are uncertain, increasing evidence indicates that hypoxia can result in a significant increase in intracellular reactive oxygen species concentration ($[ROS]_i$) through the mitochondrial electron-transport chain in pulmonary artery smooth muscle cells (PASMCs). The increased mitochondrial ROS subsequently activate protein kinase C-ε (PKCε) and NADPH oxidase (Nox), providing positive mechanisms that further increase $[ROS]_i$. ROS may directly cause extracellular Ca^{2+} influx by inhibiting voltage-dependent K^+ (K_V) channels and opening of store-operated Ca^{2+} (SOC) channels, as well as intracellular Ca^{2+} release by activating ryanodine receptors (RyRs), leading to an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and associated contraction. In concert with ROS, PKCε may also affect K_V channels, SOC channels, and RyRs, contributing to hypoxic Ca^{2+} and contractile responses in PASMCs. *Antioxid. Redox Signal.* 11, 611–623.

Introduction

It is well known that pulmonary arteries constrict in response to hypoxic exposure (<60 mm Hg Po_2). Hypoxia-induced pulmonary vasoconstriction serves as an important physiologic process that preserves the sufficient matching of regional alveolar ventilation and pulmonary perfusion in the lungs, thereby allowing sufficient oxygenation of the blood. In contrast, systemic arteries normally do not contract or even dilate in response to hypoxia to retain fairly constant blood flow to fulfill cellular metabolic demand in important organs. Despite having a unique physiologic significance, hypoxic pulmonary vasoconstriction, if sustained, may serve as a key pathologic factor leading to pulmonary hypertension and even heart failure.

The cellular and molecular mechanisms underlying the unique hypoxic pulmonary vasoconstriction and associated pulmonary hypertension remain largely elusive; however, we and many other investigators recently provided extensive evidence showing that hypoxia results in a large increase in intracellular reactive oxygen species concentration ($[ROS]_i$) in pulmonary artery smooth muscle cells (PASMCs) (10, 26, 34, 43, 47, 61, 69, 70, 97, 98, 102, 103), which is consistent with the contribution of ROS to the initiation or maintenance or both of numerous physiologic and pathologic cellular responses in virtually all types of cells. It also should be noted that the hypoxic decrease in $[ROS]_i$ has been reported (4, 49, 50, 53). Intracellular ROS can be generated by multiple resources, including the mitochondrial electron-transport chain (ETC), NADPH oxidase (Nox), xanthine oxidase, cyclooxygenase, and cytochrome P450. Among these resources, the mitochondrial ETC and Nox (4, 37, 47, 50, 61, 69, 70, 97, 102, 104, 109) have been shown to be essential for the hypoxic increase or decrease in $[ROS]_i$ in PASMCs.

A number of publications suggest that the hypoxic increase or decrease in $[ROS]_i$ can directly affect the activity of ion channels, leading to a large increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in PASMCs. For instance, hypoxia may inhibit voltage-dependent K^+ (K_V) channels by affecting $[ROS]_i$ (4, 20, 50). Presumably, hypoxic inhibition of K_V channels would result in membrane depolarization, activation of voltage-dependent Ca^{2+} (Ca_V) channels, and extracellular Ca^{2+} influx, resulting in an increase in $[Ca^{2+}]_i$. ROS also may activate ryanodine receptors/Ca^{2+}-release channels (RyRs) to induce Ca^{2+} release from the sarcoplasmic reticulum (SR), contributing to the hypoxic increase in $[Ca^{2+}]_i$ in PASMCs (40, 66). As an increase in $[Ca^{2+}]_i$ is a most important factor for cell contraction, recent studies have demonstrated that the hypoxic increase in $[Ca^{2+}]_i$ and contraction are intimately related in PASMCs (66, 72, 73). Pharmacologic and genetic interventions that inhibit or eliminate the hypoxic increase in $[Ca^{2+}]_i$ can correspondingly inhibit or eliminate

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the hypoxic contraction (38, 69, 70, 122). Moreover, hypoxia normally causes neither an increase in $[Ca^{2+}]_i$ nor a contraction in systemic (*e.g.*, cerebral and mesenteric) artery SMCs (69, 91, 99). In addition to the direct effect, ROS also may activate intermediate signaling molecules, such as protein kinase C-ε (PKCε), to regulate specific ion channels in concert with ROS, contributing to the hypoxic increase in $[Ca^{2+}]_i$ and associated contraction in PASMCs (69). It is interesting to note that our recent work revealed that mitochondrial ROS-dependent activation of PKCε can significantly augment Nox activity and lead to a further increase in intracellular ROS generation, which provides a positive-feedback mechanism to augment intracellular ROS generation further, contributing to the hypoxic increase in $[ROS]_i$ and $[Ca^{2+}]_i$ as well (70).

In this review, we summarize recent progress in the study of signaling mechanisms underlying the hypoxic ROS generation and attendant Ca^{2+} responses in PASMCs, particularly highlighting our own and others' latest work in the identification of specific sources, signaling cascades, and effective targets for ROS during hypoxic stimulation.

Hypoxia Causes a Significant Increase in $[ROS]_i$

ROS function as important signaling molecules mediating many physiologic and pathologic processes in virtually all types of cells. To explore the potential important role of ROS in hypoxic responses in PASMCS, Archer and his colleagues (4) examined the effect of hypoxia on intracellular ROS generation in isolated rat lungs by using lucigenin, a chemiluminescence reagent that is often used to assess superoxide anion (O_2^-) production. As shown in Table 1, they found that an acute hypoxic exposure for minutes causes a significant decrease in lucigenin-derived chemiluminescence, indicating a decrease in $[ROS]_i$. By using lucigenin chiefly for measuring O_2^- generation, dichlorodihydrofluorescein (H_2DCF) for hydrogen peroxide (H_2O_2), dihydroethidium for O_2^-, AmplexRed for H_2O_2 (or a combination of these), their associated research groups further confirmed findings that acute hypoxia for minutes decreases $[ROS]_i$ in freshly isolated rat pulmonary arteries and PASMCs (50), and hypoxia for hours decreases $[ROS]_i$ in passaged human PASMCs (49). Consistent with the hypoxic reduction in $[ROS]_i$, acute hypoxia results in a decrease in lucigenin-derived chemiluminescence in microsome-enriched fractions of calf pulmonary arteries (53). In contrast, with lucigenin, H_2DCF, dihydroethidium, and electron paramagnetic resonance, numerous research groups revealed that acute hypoxia increases, rather decreases, ROS generation in isolated rabbit and lamb lungs, rat and dog pulmonary arteries, and cultured calf, dog, and rat PASMCs (10, 26, 27, 34, 42–44, 47, 61, 97, 102, 103). Intriguingly, a study using dihydroethidium found that acute hypoxic exposure for minutes significantly and rapidly decreases, but for hours, markedly increases $[ROS]_i$ in passaged human PASMCs (114). We looked at the effect of acute hypoxia on $[ROS]_i$ in freshly isolated mouse PASMCs by using multiple approaches, including H_2DCF/DA (mainly for measuring H_2O_2), cytochrome *c* reduction assay and lucigenin (for O_2^-), and RedoxSensor Red CC-1 (for both O_2^- and H_2O_2). Our data indicate that acute hypoxia for minutes brings about a large increase in $[ROS]_i$ (69, 70, 97).

These previous controversial findings with conventional ROS-detection methods have been attributed to the use of

Table 1. Summary of Previous Reports on the Effect of Hypoxia on $[ROS]_i$ in Isolated Lungs, Pulmonary Arteries, and PASMCs

Authors	*Hypoxia*	*$[ROS]_i$*	*Preparations*	*Detection methods*	*References*
Archer *et al.*	Minutes	Decrease	Isolated rat lungs, pulmonary arteries, and PASMCs	AmplexRed, dihydroethidium, H_2DCF, Lucigenin	(4; 50)
Jernigan *et al.*	Minutes	Increase	Isolated rat pulmonary arteries	H_2DCF	(26; 27)
	Weeks	Increase			
Killilea *et al.*	Minutes	Increase	Cultured rat PASMCs	H_2DCF	(34)
Liu *et al.*	Minutes	Increase	Isolated porcine pulmonary arteries	Electron paramagnetic resonance, H_2DCF, Lucigenin	(43)
	Weeks				
Marshall *et al.*	Minutes	Increase	Cultured calf PASMCs	Lucigenin	(47)
Mehta *et al.*	Hours	Decrease	Cultured human PASMCs	Amplexred, Dihydroethidium, H_2DCF, Lucigenin	(49)
Mittal *et al.*	Weeks	Increase	Isolated mouse pulmonary arteries	Dihydroethidium	(51)
Mohazzab and Wolin	Minutes	Decrease	Microsome-enriched fractions of calf pulmonary arteries	Lucigenin	(53)
Paddenberg*et al.*	Minutes	Increase	Isolated mouse pulmonary arteries and cultured rabbit PASMCs	H_2DCF	(61)
Rathore *et al.*	Minutes	Increase	Isolated mouse PASMCs	Cytochrome *c* reduction assay, H_2DCF	(69; 70)
Wang *et al.*	Minutes	Increase	Isolated mouse PASMCs	H_2DCF, lucigenin, RedoxSensor Red CC-1	(97)
Wang *et al.*	Hours	Increase	Isolated rat pulmonary arteries	Dihydroethidium	(98)
Waypa *et al.*	Minutes	Increase	Cultured rat PASMCs	Fluorescence resonance energy transfer probe, H_2DCF	(102; 103)
Wu *et al.*	Minutes	Decrease	Cultured human PASMCs	Dihydroethidium	(114)
	Hours	Increase			

freshly isolated and cultured cells (56). Cultured cells undergo significant changes in expression levels and functional roles of hypoxia-responsible molecules in PASMCs (57, 122). Conversely, it should be noted that both the hypoxic decrease and increase in $[ROS]_i$ were observed in cultured PASMCs, as described earlier. Similar findings were made in freshly isolated PASMCs as well. Apparently, the increased generation of intracellular ROS by acute hypoxia in cultured PASMCs is not due to the cell culture *per se*; rather, they still retain indispensable parts of the hypoxia-sensing machinery. A concern also is expressed about the experimental findings in isolated lungs, because lungs are composed of a variety of cell types, which may produce distinct responses to hypoxic stimulation (78). However, this cannot well explain the observed hypoxic decrease and increase in $[ROS]_i$ in isolated lungs. Despite "everyone can be right" (100) or "ROS up, no way" (106), a recent report of using a novel, ratiometric, redox-sensitive fluorescence resonance energy-transfer probe demonstrates that acute hypoxia augments ROS signaling in isolated rat PASMCs (103). Consistent with this report, by using the newly developed, specific H_2O_2 biosensor HyPer (9), our more recent study reveals that acute hypoxia results in an increase in H_2O_2 generation in isolated mouse PASMCs (36). Nevertheless, a general agreement exists that chronic hypoxia increases $[ROS]_i$ in lungs, pulmonary arteries, and PASMCs (26, 27, 42, 51, 114). $[ROS]_i$ is most likely to be increased, playing an essential role in hypoxic responses in PASMCs.

Mitochondrial Electron-chain Transport Serves as a Primary Hypoxic Sensor That Initiates ROS Generation, Leading to an Increase in $[ROS]_i$

In vascular SMCs, one of the major resources for intracellular ROS generation is the mitochondrial ETC, wherein ROS can be generated at complex I, II, and III, with complex III appearing to be the main site. Archer and his colleagues (4, 50) reported that the complex I inhibitor rotenone and complex III postubisemiquinone site–inhibitor antimycin-A mimic and subsequently block the acute hypoxic decrease in $[ROS]_i$ in isolated rat lungs and PASMCs (4, 50). Conversely, Waypa *et al.* (102–104) showed that rotenone and the complex III preubisemiquinone-site inhibitor myxothiazol block, but do not mimic, the acute hypoxic responses in cultured rat PASMCs. These investigators also found that antimycin-A neither mimics nor inhibits the hypoxic effect. Similar observations were obtained in isolated rat pulmonary arteries (37) and rabbit lungs (109). In support, our recent studies reveal that multiple, structurally distinctive complex I inhibitor rotenone and methylphenylpyridinium iodide, complex II inhibitor nitropropionic acid and tenoyltrifluoroacetone, as well as the complex III preubisemiquinone-site inhibitor myxothiazol all do not mimic, but significantly block, the acute hypoxic increase in $[ROS]_i$ in freshly isolated mouse PASMCs (69, 70, 97). Moreover, antimycin-A and the complex IV inhibitor sodium azide neither mimic nor block the acute hypoxic response. The preventive effect of the complex I, II, and III preubisemiquinone-site inhibitors, but not the complex III postubisemiquinone-site and complex IV inhibitors, on the acute hypoxic increase in $[ROS]_i$ also were observed in vascular cells of isolated mouse lung slices (61). Collectively, the mitochondrial ETC molecules before the complex III ubisemiquinone site may act as a functional unit that serves to increase generation of ROS in PASMCs.

To complement pharmacologic studies, we and other investigators have begun to look at the effect of genetic inhibition of mitochondrial ROS generation on the hypoxic response. In mitochondria, O_2^- is rapidly converted to H_2O_2 by manganese superoxide dismutase; H_2O_2 is then degraded by glutathione peroxidase-1 (Gpx1) in mitochondria and the cytosol, as well as by catalase in the cytosol. Perceptibly, overexpression and deletion of these endogenous antioxidant molecules may specifically modify intracellular ROS levels and associated hypoxic responses in PASMCS. In agreement with this view, our recent study reveals that *Gpx1* gene overexpression to augment ROS removal attenuates the acute hypoxic increase in $[ROS]_i$ in freshly isolated mouse PASMCs, whereas *Gpx1* gene deletion to prevent ROS removal has the opposite effect (97). Similarly, adenoviral overexpression of mitochondrial catalase and Gpx1 attenuate the acute hypoxia-induced changes in the ROS signaling in cultured rat PASMCs (103). We also found that the hypoxic response is inhibited in PASMCs from mice with catalase gene overexpression (97).

Further to provide evidence for the initial role of mitochondria in the hypoxic increase in $[ROS]_i$ in PASMCs, we examined and compared the acute hypoxic increase in ROS generation in mitochondrial and nonmitochondrial areas of freshly isolated mouse PASMCs by using the specific mitochondrial marker MitoTracker and ROS-sensitive fluorescent dye H_2DCF. The results are shown in Fig. 1, indicating that the acute hypoxic increase in ROS generation occurs significantly earlier in mitochondrial areas than in nonmitochondrial areas. Additionally, the hypoxic increase in ROS generation is greater in the former areas than in the latter (97). We also recently showed that acute hypoxia results in a large increase in ROS generation in isolated mitochondria from mouse PASMCs (36). These findings further suggest that the mitochondrial ETC is an important primary hypoxic sensor that initiates ROS generation, leading to an increase in mitochondrial ROS generation ($[ROS]_m$) and then $[ROS]_i$ in PASMCs.

NADPH Oxidase Is Involved in the Hypoxic Increase in $[ROS]_i$

NADPH oxidase (Nox) is believed to be another important source for the generation of intracellular ROS in vascular SMCs. The active form of Nox is normally composed of various subunits, dependent on the cell type. In phagocytic cells, Nox is well characterized to include the membrane-bound subunits $p22^{phox}$ and $gp91^{phox}$ (Nox2) subunits, as well as the cytosolic subunits $p47^{phox}$ and $p67^{phox}$; the association of these cytosolic and membrane-bound subunits is required for the assembly of the active Nox. Previous studies with RT-PCR showed mRNA expression of $gp91^{phox}$, $p22^{phox}$, $p47^{phox}$, $p67^{phox}$, as well as the $gp91^{phox}$ analogues Nox1 and Nox4 in mouse lung tissues (51) and Nox4 in rabbit lungs (110). Immunofluorescence staining shows the presence of Nox4 protein in isolated human pulmonary arteries and cultured human PASMCs (85), as well as in human lung tissues (51). The existence of Nox 4 in human lungs has been shown by Western blotting (51). With Western blot analysis, we recently showed that the well-characterized, major phagocytic Nox membrane-bound subunit $p22^{phox}$, as well as the cytosolic subunits $p47^{phox}$ and $p67^{phox}$, are expressed in

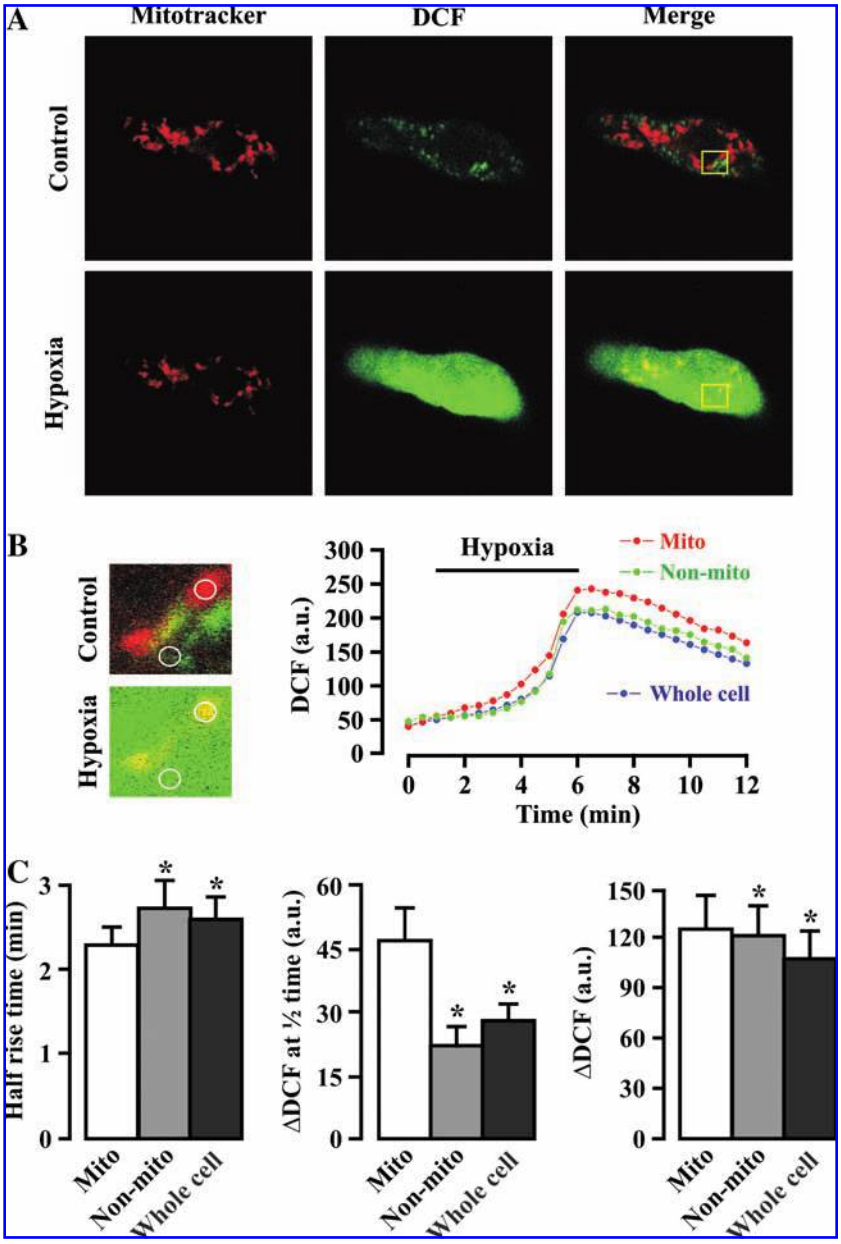

FIG. 1. Hypoxia-induced increase in ROS generation in mitochondria precedes that in nonmitochondrial areas in freshly isolated mouse pulmonary artery smooth muscle cells. (**A**) Original images show MitoTracker Deep Red 633 staining (shown as red) and DCF fluorescence (green) in a myocyte before and after hypoxia for 5 min. The superimposition of both MitoTracker staining and DCF fluorescence images produced yellow, indicating the hypoxic increase in ROS generation in mitochondria. (**B**) Extracted images were taken from the area indicated by the box in the cell shown in (**A**). The mitochondrial and nonmitochondrial area taken to measure the hypoxic increase in DCF fluorescence in the extracted images is indicated by a *circle*. Traces show the time course for the hypoxic response in mitochondrial (Mito), nonmitochondrial (Non-mito), and whole-cell areas. (**C**) Bar graph summarizes the mean half-rise time, amplitude of hypoxic increase in DCF fluorescence at the half-rise time (ΔDCF at half time), and maximal hypoxic increase in DCF fluorescence (ΔDCF) in mitochondrial, nonmitochondrial, and whole-cell areas. Data were obtained from 10 cells from five independent experiments. $*p < 0.05$ compared with mitochondrial areas. The figure is cited with permission from Wang *et al.* (97). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

endothelium-denuded mouse pulmonary arteries. Although the phagocytic Nox membrane-bound subunit $gp91^{phox}$ protein expression is not detected in pulmonary and mesenteric arteries, its analogues, Nox1 and Nox4 proteins, are observed (70).

Our study also demonstrated that acute hypoxia for minutes causes an increase in Nox activity and translocation of $p47^{phox}$, a major component of Nox, from the cytosol to the plasma membrane in endothelium-denuded mouse pulmonary arteries (70). These results suggest that Nox may contribute to the hypoxic ROS generation in PASMCs. In favor of this view, treatment with a Nox inhibitor apocynin blocks the hypoxic increase in $[ROS]_i$ in freshly isolated mouse PASMCs and Nox activity in mouse pulmonary arteries. The hypoxic

increase in $[ROS]_i$ and Nox activity are significantly prevented as well in $p47^{phox-/-}$ mouse PASMCs (Fig. 2) (70). In support of this, the Nox inhibitor diphenyleneiodonium also inhibits the hypoxic increase in $[ROS]_i$ in cultured calf PASMCs (47). Nox is another important source for the hypoxic generation of intracellular ROS in PASMCs. However, it should be noted that hypoxia may inhibit the Nox-dependent generation of intracellular ROS in a microsome-enriched fraction of calf pulmonary arteries (52).

Role of NADPH Oxidase in the Hypoxic Increase in $[ROS]_i$ Is Mediated by the Mitochondrial ROS–Protein Kinase C-ε Signaling Axis

Protein kinase C (PKC) can activate Nox to increase $[ROS]_i$, participating in a variety of cellular responses in vascular SMCs (81, 101). The PKC family consists of 12 isoforms, which can be categorized into three groups based on their structure and activation *in vitro*: the conventional PKCs (α, β_1, β_2, and γ) that are sensitive to Ca^{2+} and diacylglycerol (DAG); novel PKCs (δ, ε, η, θ, μ, and υ) that are sensitive only to DAG; and atypical PKCs (ζ and ι) that are sensitive to neither Ca^{2+} nor DAG. Damron *et al.* (17) reported that PKC isoforms (PKCα, PKCδ, PKCε, PKCζ, PKCι, and PKCυ) are expressed in cultured canine PASMCs. Our recent data reveal that PKCε protein is expressed in endothelium-denuded mouse pulmonary arteries; acute hypoxia for minutes significantly augments the total activity of PKC and PKCε (69). We more recently found that pharmacologic and genetic inhibition of PKCε blocks the hypoxia-induced increase in $[ROS]_i$ in freshly isolated mouse PASMCs (70). These findings suggest that the Nox-dependent intracellular ROS generation may be mediated by PKCε.

In support of the role of PKCε in Nox-dependent ROS generation, we showed that the conventional/novel PKC inhibitor chelerythrine and specific PKCε peptide inhibitor block the hypoxic increase in Nox activity in mouse pulmonary arteries, whereas the conventional PKC blocker Gö6796 has no effect. Our recent data further reveal that the hypoxic activation of Nox is prevented in $PKC\varepsilon^{-/-}$ mouse pulmonary arteries, and PKCε activation with PMA mimics the hypoxic response, leading to an increase of Nox activity in pulmonary arteries. This is the first report demonstrating the PKCε-dependent Nox activation as a mediator of hypoxic-induced increase in $[ROS]_i$ in PASMCs.

As the mitochondrial ETC may function as a primary oxygen sensor in the initiation of hypoxic ROS generation, we explored whether the role of PKCε is secondary to the increased generation of mitochondrial ROS and unveiled that pharmacologic inhibition of mitochondrial ROS generation with rotenone and myxothiazol both significantly prevent acute hypoxia inducing an increase in PKCε activity in mouse pulmonary arteries (69). Overexpression of Gpx1 to enhance ROS removal in mitochondria and the cytosol significantly inhibits the acute hypoxic increase in Nox activity, whereas *Gpx1* gene deletion has the opposite effect. Consistent with these results, exogenous application of H_2O_2 mimics the hypoxic response, bringing about an increase in PKCε activity. These data, together with the findings that specific inhibition of PKCε activation by pharmacologic agents and gene deletion abolishes the hypoxic activation of Nox and associated ROS generation, emphasize that the acute hypoxia-induced, Nox-dependent ROS generation is secondary to the mitochondrial ROS–PKCε signaling axis, which provides a unique positive-feedback mechanism contributing to the hypoxic increase in $[ROS]_i$ in PASMCs.

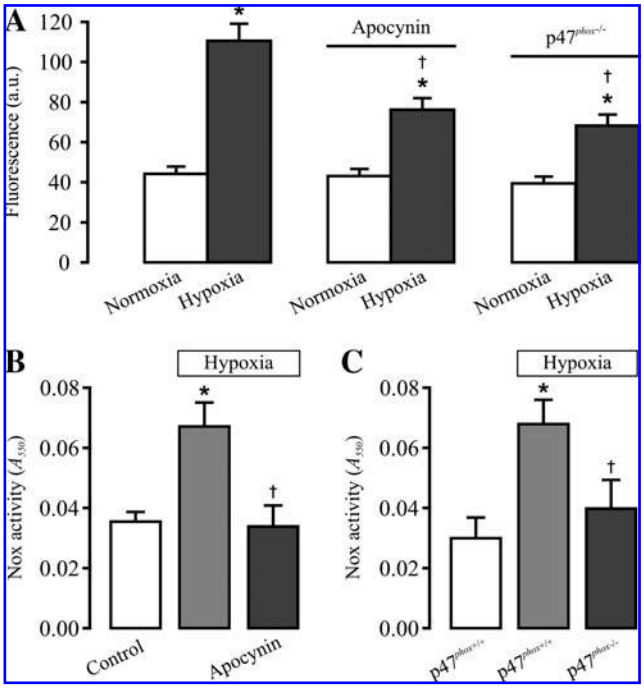

FIG. 2. Pharmacologic and genetic inhibition of NADPH oxidase significantly attenuates the hypoxic increase in $[ROS]_i$ in freshly isolated mouse pulmonary artery smooth muscle cells. (**A**) Effects of the Nox inhibitor apocynin and $p47^{phox}$ gene deletion on hypoxic increase in $[ROS]_i$ (DCF fluorescence). DCF fluorescence was recorded before (normoxia) and after hypoxia for 5 min in control ($p47^{phox+/+}$) cells, in $p47^{phox+/+}$ cells pretreated with apocynin (1 μ*M*) for 10 min, and in $p47^{phox-/-}$ cells. Data are presented as mean ± SEM from 21 to 23 cells in four independent experiments. $^*p < 0.05$ compared with normoxia (before hypoxia); $^\dagger p < 0.05$ compared with hypoxia alone. (**B**) Effects of apocynin on the hypoxic increase in Nox activity in mouse pulmonary arteries. The activity of Nox was determined in arteries treated with normoxia, hypoxia for 5 min, and apocynin (1 μ*M*) for 10 min plus hypoxia for 5 min. Data are presented as mean ± SEM from three independent experiments. $^*p < 0.05$ compared with control (normoxia); $^\dagger p < 0.05$ compared with hypoxia. (**C**) Effects of $p47^{phox}$ gene deletion on the hypoxic increase in Nox activity. Data are presented as mean ± SEM from three independent experiments. $^*p < 0.05$ compared with control (normoxia, $p47^{phox+/+}$); $^\dagger p < 0.05$ compared with hypoxia ($p47^{phox+/+}$). The figure is cited with permission from Rathore *et al.* (70).

An Increase in $[ROS]_i$ through the Mitochondrial ROS–PKCε–Nox Signaling Axis Is Critical for the Hypoxic Increase in $[Ca^{2+}]_i$ and Associated Contraction in PASMCs

In agreement with the concept that an increase in $[ROS]_i$ is essential for hypoxic responses in PASMCs, exogenous application of H_2O_2 for minutes, similar to acute hypoxia, induces an increase in $[Ca^{2+}]_i$ in cultured rat PASMCs (40, 104) and isolated rat pulmonary arteries (66). Exogenous O_2^- and

H_2O_2 cause pulmonary vasoconstriction in isolated rat pulmonary arteries as well (11, 30, 31, 35, 63, 66, 71, 77, 80, 113, 116). However, H_2O_2 also was found to dilate isolated calf pulmonary arteries (12).

Parallel to the effect on $[ROS]_i$, earlier studies by Archer's group (4, 50) reported that application of rotenone or antimycin-A mimics and then blocks the acute hypoxic contraction in isolated rat pulmonary arteries and lungs. Waypa *et al.* (102, 104) found that rotenone and myxothiazol, but not antimycin A, block the acute hypoxia-induced vasoconstriction in isolated rat lungs, and contraction as well as increase in $[Ca^{2+}]_i$ in cultured rat PASMCs; however, these inhibitors do not mimic the acute hypoxic responses (102, 104). Other research groups also discovered that rotenone or myxothiazol produces a similar inhibitory effect on the acute hypoxic contraction in isolated rat pulmonary arteries (37) and rabbit lungs (109). Our recent work indicates that various, structurally different mitochondrial complex I, II, and III preubisemiquinone-site inhibitors, including rotenone and methylphenylpyridinium iodide, nitroproprionic acid, tenoyltrifluoroacetone, and myxothiazol, all block, but do not reproduce the hypoxic increase in $[Ca^{2+}]_i$ and associated contraction in freshly isolated mouse PASMCs (69, 70, 97).

Interestingly, we found that pharmacologic inhibition of the complex I and II with tenoyltrifluoroacetone and tenoyltrifluoroacetone, complex I and III with methylphenylpyridinium iodide and myxothiazol, and complex II and III with tenoyltrifluoroacetone and myxothiazol do not produce an additive inhibitory effect on the acute hypoxic increase in $[Ca^{2+}]_i$ in mouse PASMCs (97). These findings further support the view that, in response to hypoxia, the mitochondrial complex molecules before the ubisemiquinone site in the complex III may operate as a functional unit to increase mitochondrial ROS generation, leading to an increase in $[ROS]_i$ and $[Ca^{2+}]_i$, as well as contraction in PASMCs.

Complementing these pharmacologic effects, a previous report showed that adenoviral overexpression of mitochondrial or cytosolic Gpx1 (or both) and catalase attenuate the acute hypoxic increase in $[Ca^{2+}]_i$ in cultured rat PASMCs (103). We also found that *Gpx1* gene overexpression to promote ROS removal inhibits the acute hypoxic increase in $[Ca^{2+}]_i$ and contraction in freshly isolated mouse PASMCs, whereas *Gpx1* gene deletion to inhibit ROS removal has the opposite effect. Catalase gene overexpression to enhance intracellular ROS clearance produces an inhibitory effect as well (97).

Our comparable study revealed that inhibition of PKCε with the conventional/novel PKC inhibitor chelerythrine or specific peptide inhibitor not only significantly diminishes the acute hypoxic increase in $[ROS]_i$, but also attenuates the hypoxic increase in $[Ca^{2+}]_i$ and contraction in mouse PASMCs; the hypoxic ROS, Ca^{2+}, and contractile responses are all blocked in PKC$\varepsilon^{-/-}$ mouse PASMCs as well (69). In support, numerous previous reports also showed that the PKC inhibitors H7, bisindolylmaleimide, calphostin C, and chelerythrine prevent, whereas the PKC activators PMA, thymelation, and farnesylthiotriazole mimic and subsequently block the acute hypoxic vasoconstriction in isolated canine and rabbit lungs, as well as isolated rat pulmonary arteries (8, 28, 60, 89, 111). Furthermore, the acute hypoxic vasoconstriction is inhibited in isolated lungs from PKC$\varepsilon^{-/-}$ mice (41). Consistent with the role of PKCε as a signaling molecule downstream of mitochondrial ROS, H_2O_2-induced pulmonary vasoconstriction in isolated rat pulmonary arteries has been found to be blocked by PKC inhibitors (29).

Similar to the inhibition of mitochondrial ETC and PKCε activity, pharmacologic inhibition of Nox by DPI has been found to block comparably the acute hypoxic increase in $[ROS]_i$ in PASMCs and vasoconstriction in isolated pulmonary arteries (47). Moreover, a number of publications show that Nox inhibition by DPI, iodonium diphenyl, and aminoethylbenzenesulfonyl fluoride all reduce the acute hypoxia-induced increase in $[Ca^{2+}]_i$ and contraction in cultured rat PASMCs (118), and vasoconstriction in isolated calf pulmonary arteries (52) and in rabbit and rat lungs (22, 88, 110). However, the specificity of iodonium compounds as Nox inhibitors has been disputed, because these agents can inhibit the mitochondrial ETC in heart cells and voltage-dependent Ca^{2+} currents in PASMCs (68, 107). Whereas gp91$^{phox-/-}$ mice show normal or reduced acute hypoxic responses (6, 42, 44), acute hypoxic vasoconstriction is inhibited in p47$^{phox-/-}$ mice (112). We recently demonstrated that apocynin, a more-specific Nox blocker, significantly reduces the acute hypoxic increase in $[Ca^{2+}]_i$ in freshly isolated mouse PASMCS, and p47^{phox} gene deletion produces a similar inhibitory effect (70). These results further support the view that the Nox-dependent increase in intracellular ROS generation contributes to the hypoxic increase in $[Ca^{2+}]_i$ and contraction in PASMCs.

ROS-dependent, Hypoxic Increases in $[Ca^{2+}]_i$ and Associated Contraction Are Mediated by Multiple Ion Channels in PASMCs

ROS-dependent, hypoxic increases in $[Ca^{2+}]_i$ and associated contraction in PASMCs are likely to be mediated by multiple ion channels, particularly K_V channels, SOC channels, and RyRs/Ca^{2+} release channels. Major recent advances in our understanding of the role of these ion channels in hypoxic Ca^{2+} and contractile responses are reviewed.

Involvement of voltage-dependent K^+ channels

K_V channels are important for control of the membrane potential and intracellular Ca^{2+} homeostasis, thereby playing a significant role in the regulation of vascular cell contraction. Extensive publications demonstrate that both acute and chronic hypoxia significantly inhibit K_V channels in PAMSCs, which may cause membrane depolarization, Ca_V channel opening, and extracellular Ca^{2+} influx, mediating the hypoxic increase in $[Ca^{2+}]_i$ and associated contraction (3, 48, 84). Rather surprisingly, no patch-clamp studies directly examine the effect of hypoxia on Ca_V channels in the cultured or freshly isolated rat, human, or mouse PASMCs. It also was noted that the hypoxic increase in $[Ca^{2+}]_i$ and associated contraction in PASMCs are preserved in the presence of K_V channel blockers, Ca_V channel blockers, and high extracellular K^+, as well as in the absence of extracellular Ca^{2+} (under conditions in which Ca^{2+} influx through Ca_V channels is eliminated) (18, 19, 23, 73, 79, 82).

K_V channels normally consist of α and β subunits. The α subunits form an actual ion-conducting pore, whereas the β subunits do not conduct ions on their own, but rather modulate the channel activity. Based on sequence homology of the hydrophobic transmembrane domains, the K_V channel α subunits can be divided into 12 classes, designated K_V1 to

12. $K_V\alpha$1.1, 1.2, 1.5, 1.6, 2.1, 4.3, and 9.3 channels have been shown to be hypoxia sensitive in functional activity or expression level or both, potentially participating in hypoxic responses in PASMCs (5, 7, 16, 24, 62, 64, 67, 93, 96). It is worth pointing out that previous studies that tried to determine the molecular identity of hypoxia-sensitive K_V channel members in PASMCs yielded conflicting results. Archer *et al.* (7) suggested that acute hypoxic inhibition of K_V currents in rat PASMCs is primarily caused by K_V2.1 block, but not K_V1.5, although they later reported that K_V1.5 plays a key role in acute hypoxic inhibition of K_V currents (5, 67). A study using anti-K_V2.1 antibody indicates that K_V2.1 channels may be a major hypoxic target in rat PASMCs (24). However, other investigators have not been able to detect K_V1.5 mRNA and K_V2.1 protein in rabbit or rat PASMCs (16, 62).

It has been reported that rotenone and antimycin-A mimic and subsequently inhibit the acute hypoxia-induced $[ROS]_i$ reduction, K_V-current inhibition, and contraction in isolated rat PASMCs (4, 50). Intriguingly, a recent study also showed that the multiple mitochondrial complex inhibitors attenuate K_V currents and shift K_V current activation to more-negative membrane voltages in rat PASMCs (20). Complementing the effect of mitochondrial inhibitors, a membrane permeable to hydrogen peroxide, *t*-butyl hydroperoxide, was found to inhibit K_V currents in rat PASMCs (13). These results suggest that K_V channels may be involved in ROS-dependent, hypoxic increase in $[Ca^{2+}]_i$ and contraction in PASMCs.

However, it was reported that neither removal of extracellular Ca^{2+} nor treatment with nifedipine to block Ca_V channels inhibits H_2O_2-induced increase in $[Ca^{2+}]_i$ in cultured rat PASMCs (40). Similarly, the use of the Ca_V channel blocker verapamil or removal of extracellular Ca^{2+} does not affect H_2O_2-evoked increase in $[Ca^{2+}]_i$ and contraction in isolated rat pulmonary arteries (66). A lack of the role of extracellular Ca^{2+} removal in H_2O_2-induced contraction in pulmonary arteries also was observed by other investigators (63, 80). Further studies are needed to resolve the reported inconsistent findings and to demonstrate further the role of K_V channel inhibition in ROS-dependent, hypoxic Ca^{2+} and contractile responses in PASMCs (78, 86, 90, 100).

Evidence also indicates that hypoxia may inhibit K_V channels by activating PKCε, contributing to the hypoxic increase in $[Ca^{2+}]_i$ and to contraction in PASMCs. A previous study showed that application of 4-aminopyridine to block K_V channels significantly augments hypoxic vasoconstriction in isolated lungs from PKC$\varepsilon^{-/-}$ mice, and K_v3.1b channel protein expression is increased in PKC$\varepsilon^{-/-}$ mouse lungs (41). These results indicate that PKCε may downregulate the expression and activity of K_V channels (such as K_v3.1b), participating in hypoxic responses in PASMCs. In support of this view, a previous report showed that PKC activation with PMA inhibits K_V currents in rat PASMCs, and this inhibition can be blocked by the selective PKC inhibitor bis-indolylmaleimide (119). Inhibition of K_V currents by endothelin-1 in cultured human PASMCs is also reversed by bis-indolylmaleimide and the general PKC inhibitor staurosporine (83). Similarly, Cogolludo *et al.* (14) reported that thromboxane A_2–induced inhibition of K_V currents in isolated rat PASMCs are attenuated by the general PKC inhibitors staurosporine, calphostin C, and Gö6983; however, the effect of thromboxane A_2 is not blocked by bis-indolylmaleimide or the conventional PKC inhibitor Gö6976 and can be prevented by the selective PKCζ pseudosubstrate inhibitor (14). These investigators further showed that Gö6976 blocks serotonin-evoked inhibition of native K_V currents in rat PASMCs and human K_V1.5 currents stably expressed in LTK cells (15), and PKCζ gene deletion prevents thromboxane A_2–induced inhibition of K_V currents in isolated mouse PASMCs (54). These data further support the concept that PKCε is involved in the hypoxic inhibition of K_V channels and also suggest that PKCε and PKCζ may differentially mediate agonist-induced responses in PASMCs.

Role of store-operated Ca^{2+} channels

ROS-dependent, hypoxic increase in $[Ca^{2+}]_i$ may result from extracellular Ca^{2+} influx due to activation of SOC channels in PASMCs. Pharmacologic studies showed that pretreatment with La^{3+} to inhibit SOC channels or cyclopiazonic acid to deplete SR Ca^{2+} significantly inhibits hypoxic vasoconstriction in isolated rat pulmonary arteries (73) and rat lungs (105). Acute hypoxia also significantly increases extracellular Ca^{2+} influx *via* SOC channels in pig, rabbit, and rat PASMCs (32, 39, 45, 58, 59, 94, 95).

The major molecular candidates for SOC channels are likely to be canonic transient receptor potential (TRPC) channels. These channels include seven members named TRPC1–7, each encoded by a different gene. All seven TRPC channels have been found to be expressed in mRNA or protein levels or both in pulmonary arteries, among which, TRPC1 and TRPC6 channels are likely to be involved in the acute and chronic hypoxic increase in $[Ca^{2+}]_i$ and contraction in PAMSCs (33, 39, 95, 108).

It is interesting to note that H_2O_2-induced increase in $[Ca^{2+}]_i$ in PASMCs does not appear to be related to TRPC channels because the general channel blockers La^{3+} and SKF-96365 fail to produce an inhibitory effect in cultured rat PASMCs. In addition, H_2O_2 does not affect Mn^{2+}-induced quenching of fura-2 fluorescence, a typical indicator of the opening of TRPC-encoded SOC channels (40). Similarly, H_2O_2-evoked vasoconstriction in isolated pulmonary arteries is not affected by SKF-96365 (66). Moreover, H_2O_2-induced increases in $[Ca^{2+}]_i$ in cultured rat PASMCs and isolated rat pulmonary arteries are not inhibited by removal of extracellular Ca^{2+} (40, 66).

Despite the lack of direct experimental evidence for the effect of PKC on TRPC channels in PASMCs, previous studies reported that SOC channels in systemic vascular (*e.g.*, coronary artery, mesenteric artery, and portal vein) SMCs are activated by the PKC activators phorbol ester phorbol 12,13-dibutyrate and 1-oleoyl-2-acetyl-*sn*-glycerol, as well as a PKC catalytic subunit, whereas they are inhibited by the PKC inhibitor chelerythrine (2, 74, 75). TRPC1 channels have been shown to be phosphorylated by PKCα, regulating store-operated Ca^{2+} entry in human endothelial cells (1). Moreover, PKCα is known to participate in the activation of SOCs in mesangial cells (46). Thus, it is interesting to determine whether a similar mechanism exits in PASMCs.

Contribution of ryanodine receptors/ Ca^{2+} release channels

The Ca^{2+} release from the SR *via* RyRs is a major component of Ca^{2+} signaling in vascular SMCs. The role of RyRs in the hypoxic Ca^{2+} release and associated contraction in

PASMCs has received extensive attention. Numerous publications have shown that the depletion of SR Ca^{2+} with caffeine (through activation of RyRs) reduces or abolishes the acute hypoxia-induced increase in $[Ca^{2+}]_i$ in cultured rat and freshly isolated canine and rat PASMCs (65, 76, 99) and vasoconstriction in isolated canine and rabbit pulmonary arteries (19, 25). Similarly, ryanodine, an agent that binds with high affinity to RyRs, largely inhibits the acute hypoxic Ca^{2+} response in cultured cat PASMCs (91) and vasoconstriction in isolated rat lungs and canine and rabbit pulmonary arteries (19, 25, 55). Other RyR antagonists, such as ruthenium red, tetracaine, and dantrolene, also significantly block the acute hypoxic increase in $[Ca^{2+}]_i$ in freshly isolated rat PASMCs and vasoconstriction in pulmonary arteries (122). These data suggest that RyRs are key targets for acute hypoxia, by which hypoxia may induce Ca^{2+} release and associated contraction in PASMCs. The importance of RyR-mediated Ca^{2+} release in hypoxic responses in PASMCs is reinforced by the findings that hypoxic inhibition of K_V channels is likely to be secondary to Ca^{2+} release from the SR (21, 65, 92). Moreover, hypoxic Ca^{2+} release through RyRs may result in the opening of SOC channels, which causes not only extracellular Ca^{2+} influx through the opening channels, but also may result in membrane depolarization, activation of Ca_V channels, and further Ca^{2+} influx, providing a positive-feedback mechanism that enhances hypoxic increase in $[Ca^{2+}]_i$ and contraction in PASMCs (58, 59).

Consistent with the potentially important role of RyRs in ROS-dependent, hypoxic Ca^{2+} and contractile responses, a previous study showed that treatment with ryanodine (50 μM) to block RyRs significantly inhibits H_2O_2-evoked, initial rapid increase in $[Ca^{2+}]_i$ in cultured rat PASMCS (40). In support, ryanodine and dantrolene abolish or greatly suppress an H_2O_2-induced increase in $[Ca^{2+}]_i$ and vasoconstriction in isolated rat pulmonary arteries (66).

Three distinct gene-encoded subtypes of RyRs (RyR1, RyR2, and RyR3) are expressed in mammalian cells. By using real-time quantitative RT-PCR, we showed that RyR1, RyR2, and RyR3 are all expressed in freshly isolated rat and mouse PASMCs (121, 122). In support of our findings, other investigators reported that all three RyR subtype mRNAs are

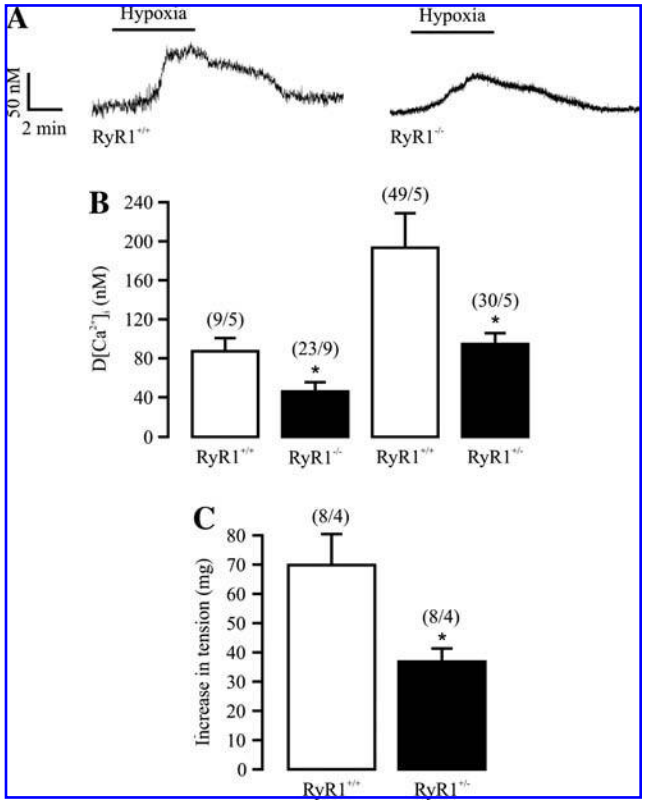

FIG. 3. RyR1 mediates the hypoxic increase in $[Ca^{2+}]_i$ and contraction in pulmonary artery smooth muscle cells. (**A**) Original recordings show that hypoxic exposure for 5 min induced an increase in $[Ca^{2+}]_i$ in an embryonic $RyR1^{+/+}$ and $RyR1^{-/-}$ mouse PASMC. (**B**) Bar graphs summarize the hypoxic increase in $[Ca^{2+}]_i$ in embryonic $RyR1^{+/+}$, embryonic $RyR1^{-/-}$, adult $RyR^{+/-}$, and adult $RyR1^{+/+}$ PASMCs. $^*p < 0.05$ compared with $RyR1^{+/+}$ cells. Numbers in parentheses indicate the numbers of cells and mice tested. (**C**) Summary of hypoxic vasoconstriction in adult $RyR1^{+/+}$ and $RyR^{+/-}$ mouse pulmonary arteries. $^*p < 0.05$ compared with $RyR1^{+/+}$ pulmonary arteries. The figure is cited with permission from Li *et al.* (38).

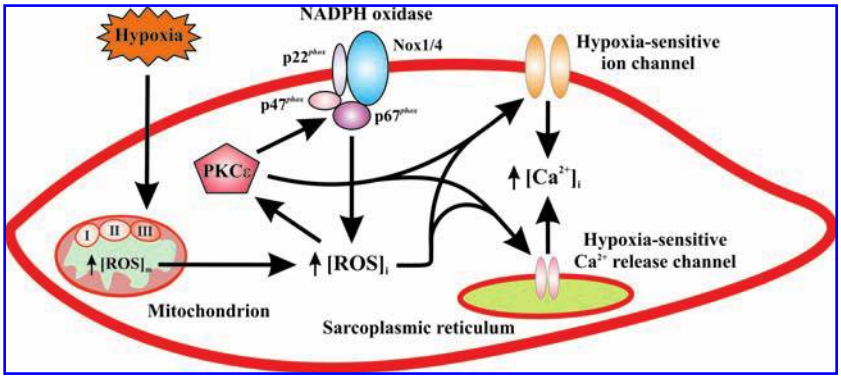

FIG. 4. A schematic diagram illustrating the signaling mechanisms for ROS-dependent, hypoxic increase in $[Ca^{2+}]_i$ and associated contraction in pulmonary artery smooth muscle cells. This includes the potential important primary hypoxic sensor mitochondrial ETC, intermediate signaling molecules ROS, PKCε, and Nox, as well as effectors hypoxia-sensitive plasmalemmal ion channels (*e.g.*, K_V channels and SOC channels) and sarcolemmal Ca^{2+} release channels (RyRs). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

present in rat intralobar pulmonary arteries (117). Expression of RyR1, RyR2, and RyR3 proteins also was observed in freshly isolated rat PASMCs by using immunofluorescence staining (122) and in isolated pulmonary arteries by using Western blot analysis (117).

We have started to explore the potential role of individual subtypes of RyRs in hypoxic responses by using genetically manipulated mice. As shown in Fig. 3, our studies revealed that acute hypoxia induces a much smaller increase in $[Ca^{2+}]_i$ in freshly isolated PASMCs from embryonic $RyR1^{-/-}$ mice at day 17, compared with wild-type mice. A decreased Ca^{2+} response also was observed in adult $RyR1^{+/-}$ mouse PASMCs. Moreover, acute hypoxic vasoconstriction is inhibited in pulmonary arteries from adult $RyR1^{+/-}$ mice (38). The acute hypoxic increase in $[Ca^{2+}]_i$ in PASMCs and vasoconstriction in pulmonary arteries are significantly diminished in adult $RyR3^{-/-}$ mice as well (122). As FK506 binding protein with a molecular mass of 12.6 kDa (FKBP12.6) is known to bind to and regulate RyR2 (115), we used $FKBP12.6^{-/-}$ mice as a unique tool in determining the potential role of RyR2 in hypoxic responses; we found that that the acute hypoxia-induced increase in $[Ca^{2+}]_i$ in PASMCs and vasoconstriction in isolated pulmonary arteries are both enhanced in $FKBP12.6^{-/-}$ mice (120). Collectively, all three RyR subtypes are involved in the hypoxic Ca^{2+} release and contraction in PASMCs.

It has been reported that addition of catalytic PKC phosphorylates RyR2 in canine cardiac microsomes. The observed extent of PKC-dependent phosphorylation of RyR2 is comparable to the level of PKC-dependent increase in the activity of RyR2 determined by [^{3}H]ryanodine-binding assay (87). By analogy, PKC is likely to regulate RyRs directly to mediate hypoxic Ca^{2+} release in PASMCs; however, this view must be demonstrated by further studies.

Conclusions

Based on our recent studies and previous publications, we present a schematic diagram, as illustrated in Fig. 4, to conclude that the mitochondrial ETC may function as a hypoxic sensor in PASMCs, by which hypoxia can significantly enhance $[ROS]_m$, leading to an initial, large increase in $[ROS]_i$. The increased $[ROS]_i$ subsequently activates the intermediate signaling molecules PKCε and Nox, providing a positive-feedback mechanism to increase further the hypoxic generation of intracellular ROS. As a consequence, ROS and PKCε synergistically result in the inhibition of plasmalemmal K_V channels (hypoxic effectors), opening of Ca_V channels, and extracellular Ca^{2+} influx, contributing to the hypoxic increase in $[Ca^{2+}]_i$ and associated contraction. In addition, ROS and PKCε may activate plasmalemmal SOC channels. As important hypoxic effectors, the opened SOC channels not only allow extracellular Ca^{2+} to enter the cell, but also cause membrane depolarization, leading to the further opening of Ca_V channels and extracellular Ca^{2+} influx. Moreover, both ROS and PKCε can in concert activate RyR1, RyR2, RyR3 or all three, inducing Ca^{2+} release from the SR, as an important process for the hypoxic Ca^{2+} and contractile responses in PASMCs. Interestingly, available evidence suggests that the hypoxic inhibition of K_V channels and activation of SOC channels are likely to be secondary to SR Ca^{2+} release. Finally, it is worth noting that, despite recent progress in the field, we are still far from fully understanding the cellular and molecular mechanisms responsible for hypoxic increases in $[Ca^{2+}]_i$ and associated contraction in PASMCs. For instance, it is unclear how the mitochondrial ETC senses hypoxia in PASMCs. To what extent each of the individual ion channels contributes to the hypoxic Ca^{2+} response remains to be determined. Thus, further studies are necessary to answer these fundamental questions and also to resolve the reported inconsistent findings.

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References

1. Ahmmed GU, Mehta D, Vogel S, Holinstat M, Paria BC, Tiruppathi C, and Malik AB. Protein kinase Calpha phosphorylates the TRPC1 channel and regulates store-operated Ca^{2+} entry in endothelial cells. *J Biol Chem* 279: 20941–20949, 2004.
2. Albert AP and Large WA. Activation of store-operated channels by noradrenaline via protein kinase C in rabbit portal vein myocytes. *J Physiol* 544: 113–125, 2002.
3. Archer SL, Gomberg-Maitland M, Maitland ML, Rich S, Garcia JG, and Weir EK. Mitochondrial metabolism, redox signaling, and fusion: a mitochondria-ROS-HIF-1α-Kv1.5 O_2-sensing pathway at the intersection of pulmonary hypertension and cancer. *Am J Physiol Heart Circ Physiol* 294: H570–H578, 2008.
4. Archer SL, Huang J, Henry T, Peterson D, and Weir EK. A redox-based O_2 sensor in rat pulmonary vasculature. *Circ Res* 73: 1100–1112, 1993.
5. Archer SL, London B, Hampl V, Wu X, Nsair A, Puttagunta L, Hashimoto K, Waite RE, and Michelakis ED. Impairment of hypoxic pulmonary vasoconstriction in mice lacking the voltage-gated potassium channel Kv1.5. *FASEB J* 15: 1801–1803, 2001.
6. Archer SL, Reeve HL, Michelakis E, Puttagunta L, Waite R, Nelson DP, Dinauer MC, and Weir EK. O_2 sensing is preserved in mice lacking the $gp91^{phox}$ subunit of NADPH oxidase. *Proc Natl Acad Sci U S A* 96: 7944–7949, 1999.
7. Archer SL, Souil E, Dinh-Xuan AT, Schremmer B, Mercier JC, El Yaagoubi A, Nguyen-Huu L, Reeve HL, and Hampl V. Molecular identification of the role of voltage-gated K^+ channels, Kv1.5 and Kv2.1, in hypoxic pulmonary vasoconstriction and control of resting membrane potential in rat pulmonary artery myocytes. *J Clin Invest* 101: 2319–2330, 1998.
8. Barman SA. Potassium channels modulate canine pulmonary vasoreactivity to protein kinase C activation. *Am J Physiol* 277: L558–L565, 1999.
9. Belousov VV, Fradkov AF, Lukyanov KA, Staroverov DB, Shakhbazov KS, Terskikh AV, and Lukyanov S. Genetically encoded fluorescent indicator for intracellular hydrogen peroxide. *Nat Methods* 3: 281–286, 2006.
10. Brennan LA, Steinhorn RH, Wedgwood S, Mata-Greenwood E, Roark EA, Russell JA, and Black SM. Increased

superoxide generation is associated with pulmonary hypertension in fetal lambs: a role for NADPH oxidase. *Circ Res* 92: 683–691, 2003.
11. Burghuber OC, Strife R, Zirolli J, Mathias MM, Murphy RC, Reeves JT, and Voelkel NF. Hydrogen peroxide induced pulmonary vasoconstriction in isolated rat lungs is attenuated by U60,257, a leucotriene synthesis blocker. *Wien Klin Wochenschr* 98: 117–119, 1986.
12. Burke TM and Wolin MS. Hydrogen peroxide elicits pulmonary arterial relaxation and guanylate cyclase activation. *Am J Physiol* 252: H721–H732, 1987.
13. Cogolludo A, Frazziano G, Cobeno L, Moreno L, Lodi F, Villamor E, Tamargo J, and Perez-Vizcaino F. Role of reactive oxygen species in Kv channel inhibition and vasoconstriction induced by TP receptor activation in rat pulmonary arteries. *Ann N Y Acad Sci* 1091: 41–51, 2006.
14. Cogolludo A, Moreno L, Bosca L, Tamargo J and Perez-Vizcaino F. Thromboxane A_2-induced inhibition of voltage-gated K^+ channels and pulmonary vasoconstriction: role of protein kinase Cζ. *Circ Res* 93: 656–663, 2003.
15. Cogolludo A, Moreno L, Lodi F, Frazziano G, Cobeno L, Tamargo J, and Perez-Vizcaino F. Serotonin inhibits voltage-gated K^+ currents in pulmonary artery smooth muscle cells: role of 5-HT2A receptors, caveolin-1, and KV1.5 channel internalization. *Circ Res* 98: 931–938, 2006.
16. Coppock EA and Tamkun MM. Differential expression of K_V channel alpha- and beta-subunits in the bovine pulmonary arterial circulation. *Am J Physiol Lung Cell Mol Physiol* 281: L1350–L1360, 2001.
17. Damron DS, Nadim HS, Hong SJ, Darvish A, and Murray PA. Intracellular translocation of PKC isoforms in canine pulmonary artery smooth muscle cells by ANG II. *Am J Physiol* 274: L278–L288, 1998.
18. Demiryurek AT, Wadsworth RM, Kane KA, and Peacock AJ. The role of endothelium in hypoxic constriction of human pulmonary artery rings. *Am Rev Respir Dis* 147: 283–290, 1993.
19. Dipp M, Nye PC, and Evans AM. Hypoxic release of calcium from the sarcoplasmic reticulum of pulmonary artery smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 281: L318–L325, 2001.
20. Firth AL, Yuill KH, and Smirnov SV. Mitochondria-dependent regulation of Kv currents in rat pulmonary artery smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 295: L61–L70, 2008.
21. Gelband CH and Gelband H. Ca^{2+} release from intracellular stores is an initial step in hypoxic pulmonary vasoconstriction of rat pulmonary artery resistance vessels. *Circulation* 96: 3647–3654, 1997.
22. Grimminger F, Weissmann N, Spriestersbach R, Becker E, Rosseau S, and Seeger W. Effects of NADPH oxidase inhibitors on hypoxic vasoconstriction in buffer-perfused rabbit lungs. *Am J Physiol* 268: L747–L752, 1995.
23. Hasunuma K, Rodman DM, and McMurtry IF. Effects of K^+ channel blockers on vascular tone in the perfused rat lung. *Am Rev Respir Dis* 144: 884–887, 1991.
24. Hogg DS, Davies AR, McMurray G, and Kozlowski RZ. K_V2.1 channels mediate hypoxic inhibition of I(KV) in native pulmonary arterial smooth muscle cells of the rat. *Cardiovasc Res* 55: 349–360, 2002.
25. Jabr RI, Toland H, Gelband CH, Wang XX, and Hume JR. Prominent role of intracellular Ca^{2+} release in hypoxic vasoconstriction of canine pulmonary artery. *Br J Pharmacol* 122: 21–30, 1997.
26. Jernigan NL, Resta TC, and Walker BR. Contribution of oxygen radicals to altered NO-dependent pulmonary vasodilation in acute and chronic hypoxia. *Am J Physiol Lung Cell Mol Physiol* 286: L947–L955, 2004.
27. Jernigan NL, Walker BR, and Resta TC. Reactive oxygen species mediate RhoA/Rho kinase-induced Ca^{2+} sensitization in pulmonary vascular smooth muscle following chronic hypoxia. *Am J Physiol Lung Cell Mol Physiol* 295: L515–L529, 2008.
28. Jin N, Packer CS, and Rhoades RA. Pulmonary arterial hypoxic contraction: signal transduction. *Am J Physiol* 263: L73–L78, 1992.
29. Jin N, Packer CS, and Rhoades RA. Reactive oxygen-mediated contraction in pulmonary arterial smooth muscle: cellular mechanisms. *Can J Physiol Pharmacol* 69: 383–388, 1991.
30. Jin N and Rhoades RA. Activation of tyrosine kinases in H_2O_2-induced contraction in pulmonary artery. *Am J Physiol* 272: H2686–H2692, 1997.
31. Jones RD, Thompson JS, and Morice AH. The effect of hydrogen peroxide on hypoxia, prostaglandin F2 alpha and potassium chloride induced contractions in isolated rat pulmonary arteries. *Pulmon Pharmacol Ther* 10: 37–42, 1997.
32. Kang TM, Park MK, and Uhm DY. Effects of hypoxia and mitochondrial inhibition on the capacitative calcium entry in rabbit pulmonary arterial smooth muscle cells. *Life Sci* 72: 1467–1479, 2003.
33. Keseru B, Barbosa-Sicard E, Popp R, Fisslthaler B, Dietrich A, Gudermann T, Hammock BD, Falck JR, Weissmann N, Busse R, and Fleming I. Epoxyeicosatrienoic acids and the soluble epoxide hydrolase are determinants of pulmonary artery pressure and the acute hypoxic pulmonary vasoconstrictor response. *FASEB J* 22: 4306–4315, 2008.
34. Killilea DW, Hester R, Balczon R, Babal P, and Gillespie MN. Free radical production in hypoxic pulmonary artery smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 279: L408–L412, 2000.
35. Kjaeve J, Vaage J, and Bjertnaes L. Toxic oxygen metabolites induce vasoconstriction and bronchoconstriction in isolated, plasma-perfused rat lungs. *Acta Anaesthesiol Scand* 35: 65–70, 1991.
36. Korde AS and Wang YX. Mitochondrial rieske protein, are you a real hypoxic sensor in pulmonary artery smooth muscle cells? *FASEB J* 22: 11174. 2008.
37. Leach RM, Hill HM, Snetkov VA, Robertson TP, and Ward JP. Divergent roles of glycolysis and the mitochondrial electron transport chain in hypoxic pulmonary vasoconstriction of the rat: identity of the hypoxic sensor. *J Physiol* 536: 211–224, 2001.
38. Li XQ, Zheng YM, Rathore R, Ma J, Takeshima H, and Wang YX. Genetic evidence for functional role of ryanodine receptor 1 in pulmonary artery smooth muscle cells. *Pflugers Arch* 457: 771–783, 2009.
39. Lin MJ, Leung GP, Zhang WM, Yang XR, Yip KP, Tse CM, and Sham JS. Chronic hypoxia-induced upregulation of store-operated and receptor-operated Ca^{2+} channels in pulmonary arterial smooth muscle cells: a novel mechanism of hypoxic pulmonary hypertension. *Circ Res* 95: 496–505, 2004.
40. Lin MJ, Yang XR, Cao YN, and Sham JS. Hydrogen peroxide-induced Ca^{2+} mobilization in pulmonary arterial smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 292: L1598–L1608, 2007.
41. Littler CM, Morris KG Jr, Fagan KA, McMurtry IF, Messing RO, and Dempsey EC. Protein kinase C-ε-null mice have

decreased hypoxic pulmonary vasoconstriction. *Am J Physiol Heart Circ Physiol* 284: H1321–H1331, 2003.
42. Liu JQ, Erbynn EM, and Folz RJ. Chronic hypoxia-enhanced murine pulmonary vasoconstriction: role of superoxide and gp91^{phox}. *Chest* 128: 594S–596S, 2005.
43. Liu JQ, Sham JS, Shimoda LA, Kuppusamy P, and Sylvester JT. Hypoxic constriction and reactive oxygen species in porcine distal pulmonary arteries. *Am J Physiol Lung Cell Mol Physiol* 285: L322–L333, 2003.
44. Liu JQ, Zelko IN, Erbynn EM, Sham JS, and Folz RJ. Hypoxic pulmonary hypertension: role of superoxide and NADPH oxidase (gp91^{phox}). *Am J Physiol Lung Cell Mol Physiol* 290: L2–L10, 2006.
45. Lu W, Wang J, Shimoda LA, and Sylvester JT. Differences in STIM1 and TRPC expression in proximal and distal pulmonary arterial smooth muscle are associated with differences in Ca^{2+} responses to hypoxia. *Am J Physiol Lung Cell Mol Physiol* 295: L104–L113, 2008.
46. Ma R, Kudlacek PE, and Sansom SC. Protein kinase Cα participates in activation of store-operated Ca^{2+} channels in human glomerular mesangial cells. *Am J Physiol Cell Physiol* 283: C1390–C1398, 2002.
47. Marshall C, Mamary AJ, Verhoeven AJ, and Marshall BE. Pulmonary artery NADPH-oxidase is activated in hypoxic pulmonary vasoconstriction. *Am J Respir Cell Mol Biol* 15: 633–644, 1996.
48. Mauban JR, Remillard CV, and Yuan JX. Hypoxic pulmonary vasoconstriction: role of ion channels. *J Appl Physiol* 98: 415–420, 2005.
49. Mehta JP, Campian JL, Guardiola J, Cabrera JA, Weir EK, and Eaton JW. Generation of oxidants by hypoxic human pulmonary and coronary smooth-muscle cells. *Chest* 133: 1410–1414, 2008.
50. Michelakis ED, Hampl V, Nsair A, Wu X, Harry G, Haromy A, Gurtu R, and Archer SL. Diversity in mitochondrial function explains differences in vascular oxygen sensing. *Circ Res* 90: 1307–1315, 2002.
51. Mittal M, Roth M, Konig P, Hofmann S, Dony E, Goyal P, Selbitz AC, Schermuly RT, Ghofrani HA, Kwapiszewska G, Kummer W, Klepetko W, Hoda MA, Fink L, Hanze J, Seeger W, Grimminger F, Schmidt HH, and Weissmann N. Hypoxia-dependent regulation of nonphagocytic NADPH oxidase subunit NOX4 in the pulmonary vasculature. *Circ Res* 101: 258–267, 2007.
52. Mohazzab KM, Fayngersh RP, Kaminski PM, and Wolin MS. Potential role of NADH oxidoreductase-derived reactive O_2 species in calf pulmonary arterial PO_2-elicited responses. *Am J Physiol* 269: L637–L644, 1995.
53. Mohazzab KM and Wolin MS. Properties of a superoxide anion-generating microsomal NADH oxidoreductase, a potential pulmonary artery PO_2 sensor. *Am J Physiol* 267: L823–L831, 1994.
54. Moreno L, Frazziano G, Cogolludo A, Cobeno L, Tamargo J, and Perez-Vizcaino F. Role of protein kinase Cζ and its adaptor protein p62 in voltage-gated potassium channel modulation in pulmonary arteries. *Mol Pharmacol* 72: 1301–1309, 2007.
55. Morio Y and McMurtry IF. Ca^{2+} release from ryanodine-sensitive store contributes to mechanism of hypoxic vasoconstriction in rat lungs. *J Appl Physiol* 92: 527–534, 2002.
56. Moudgil R, Michelakis ED, and Archer SL. Hypoxic pulmonary vasoconstriction. *J Appl Physiol* 98: 390–403, 2005.
57. Ng LC, Kyle BD, Lennox AR, Shen XM, Hatton WJ, and Hume JR. Cell culture alters Ca^{2+} entry pathways activated by store-depletion or hypoxia in canine pulmonary arterial smooth muscle cells. *Am J Physiol Cell Physiol* 294: C313–C323, 2008.
58. Ng LC, Wilson SM, and Hume JR. Mobilization of sarcoplasmic reticulum stores by hypoxia leads to consequent activation of capacitative Ca^{2+} entry in isolated canine pulmonary arterial smooth muscle cells. *J Physiol* 563: 409–419, 2005.
59. Ng LC, Wilson SM, McAllister CE, and Hume JR. Role of InsP3 and ryanodine receptors in the activation of capacitative Ca^{2+} entry by store depletion or hypoxia in canine pulmonary arterial smooth muscle cells. *Br J Pharmacol* 152: 101–111, 2007.
60. Orton EC, Raffestin B, and McMurtry IF. Protein kinase C influences rat pulmonary vascular reactivity. *Am Rev Respir Dis* 141: 654–658, 1990.
61. Paddenberg R, Ishaq B, Goldenberg A, Faulhammer P, Rose F, Weissmann N, Braun-Dullaeus RC, and Kummer W. Essential role of complex II of the respiratory chain in hypoxia-induced ROS generation in the pulmonary vasculature. *Am J Physiol Lung Cell Mol Physiol* 284: L710–L719, 2003.
62. Patel AJ, Lazdunski M, and Honore E. Kv2.1/Kv9.3, a novel ATP-dependent delayed-rectifier K^+ channel in oxygen-sensitive pulmonary artery myocytes. *EMBO J* 16: 6615–6625, 1997.
63. Pelaez NJ, Braun TR, Paul RJ, Meiss RA and Packer CS. H(2)O(2) mediates Ca^{2+}- and MLC_{20} phosphorylation-independent contraction in intact and permeabilized vascular muscle. *Am J Physiol Heart Circ Physiol* 279: H1185–H1193, 2000.
64. Platoshyn O, Yu Y, Golovina VA, McDaniel SS, Krick S, Li L, Wang JY, Rubin LJ, and Yuan JX. Chronic hypoxia decreases K_V channel expression and function in pulmonary artery myocytes. *Am J Physiol Lung Cell Mol Physiol* 280: L801–L812, 2001.
65. Post JM, Gelband CH, and Hume JR. $[Ca^{2+}]_i$ inhibition of K^+ channels in canine pulmonary artery: novel mechanism for hypoxia-induced membrane depolarization. *Circ Res* 77: 131–139, 1995.
66. Pourmahram GE, Snetkov VA, Shaifta Y, Drndarski S, Knock GA, Aaronson PI, and Ward JP. Constriction of pulmonary artery by peroxide: role of Ca^{2+} release and PKC. *Free Radic Biol Med* 45: 1468–1476, 2008.
67. Pozeg ZI, Michelakis ED, McMurtry MS, Thebaud B, Wu XC, Dyck JR, Hashimoto K, Wang S, Moudgil R, Harry G, Sultanian R, Koshal A, and Archer SL. In vivo gene transfer of the O_2-sensitive potassium channel Kv1.5 reduces pulmonary hypertension and restores hypoxic pulmonary vasoconstriction in chronically hypoxic rats. *Circulation* 107: 2037–2044, 2003.
68. Ragan CI and Bloxham DP. Specific labelling of a constituent polypeptide of bovine heart mitochondrial reduced nicotinamide-adenine dinucleotide-ubiquinone reductase by the inhibitor diphenyleneiodonium. *Biochem J* 163: 605–615, 1977.
69. Rathore R, Zheng YM, Li XQ, Wang QS, Liu QH, Ginnan R, Singer HA, Ho YS, and Wang YX. Mitochondrial ROS-PKCε signaling axis is uniquely involved in hypoxic increase in $[Ca^{2+}]_i$ in pulmonary artery smooth muscle cells. *Biochem Biophys Res Commun* 351: 784–790, 2006.
70. Rathore R, Zheng YM, Niu CF, Liu QH, Korde A, Ho YS, and Wang YX. Hypoxia activates NADPH oxidase to increase $[ROS]_i$ and $[Ca^{2+}]_i$ through the mitochondrial

ROS-PKCε signaling axis in pulmonary artery smooth muscle cells. *Free Radic Biol Med* 45: 1223–1231, 2008.
71. Rhoades RA, Packer CS, Roepke DA, Jin N, and Meiss RA. Reactive oxygen species alter contractile properties of pulmonary arterial smooth muscle. *Can J Physiol Pharmacol* 68: 1581–1589, 1990.
72. Robertson TP, Aaronson PI, and Ward JP. Hypoxic vasoconstriction and intracellular Ca^{2+} in pulmonary arteries: evidence for PKC-independent Ca^{2+} sensitization. *Am J Physiol* 268: H301–H307, 1995.
73. Robertson TP, Hague D, Aaronson PI, and Ward JP. Voltage-independent calcium entry in hypoxic pulmonary vasoconstriction of intrapulmonary arteries of the rat. *J Physiol* 525: 669–680, 2000.
74. Saleh SN, Albert AP, Peppiatt CM, and Large WA. Angiotensin II activates two cation conductances with distinct TRPC1 and TRPC6 channel properties in rabbit mesenteric artery myocytes. *J Physiol* 577: 479–495, 2006.
75. Saleh SN, Albert AP, Peppiatt-Wildman CM, and Large WA. Diverse properties of store-operated TRPC channels activated by protein kinase C in vascular myocytes. *J Physiol* 586: 2463–2476, 2008.
76. Salvaterra CG and Goldman WF. Acute hypoxia increases cytosolic calcium in cultured pulmonary arterial myocytes. *Am J Physiol* 264: L323–L328, 1993.
77. Seeger W, Suttorp N, Schmidt F, and Neuhof H. The glutathione redox cycle as a defense system against hydrogen-peroxide-induced prostanoid formation and vasoconstriction in rabbit lungs. *Am Rev Respir Dis* 133: 1029–1036, 1986.
78. Sham JS. Hypoxic pulmonary vasoconstriction: ups and downs of reactive oxygen species. *Circ Res* 91: 649–651, 2002.
79. Sham JS, Crenshaw BR Jr, Deng LH, Shimoda LA, and Sylvester JT. Effects of hypoxia in porcine pulmonary arterial myocytes: roles of K_V channel and endothelin-1. *Am J Physiol Lung Cell Mol Physiol* 279: L262–L272, 2000.
80. Sheehan DW, Giese EC, Gugino SF, and Russell JA. Characterization and mechanisms of H_2O_2-induced contractions of pulmonary arteries. *Am J Physiol* 264: H1542–H1547, 1993.
81. Shen GX. Selective protein kinase C inhibitors and their applications. *Curr Drug Targets Cardiovasc Haematol Disord* 3: 301–307, 2003.
82. Shimoda LA, Sham JS, Shimoda TH, and Sylvester JT. L-type Ca^{2+} channels, resting $[Ca^{2+}]_i$, and ET-1-induced responses in chronically hypoxic pulmonary myocytes. *Am J Physiol Lung Cell Mol Physiol* 279: L884–L894, 2000.
83. Shimoda LA, Sylvester JT, Booth GM, Shimoda TH, Meeker S, Undem BJ, and Sham JS. Inhibition of voltage-gated K^+ currents by endothelin-1 in human pulmonary arterial myocytes. *Am J Physiol Lung Cell Mol Physiol* 281: L1115–L1122, 2001.
84. Shimoda LA, Wang J, and Sylvester JT. Ca^{2+} channels and chronic hypoxia. *Microcirculation* 13: 657–670, 2006.
85. Sturrock A, Cahill B, Norman K, Huecksteadt TP, Hill K, Sanders K, Karwande SV, Stringham JC, Bull DA, Gleich M, Kennedy TP, and Hoidal JR. Transforming growth factor-beta1 induces Nox4 NAD(P)H oxidase and reactive oxygen species-dependent proliferation in human pulmonary artery smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 290: L661–L673, 2006.
86. Sylvester JT. Hypoxic pulmonary vasoconstriction: a radical view. *Circ Res* 88: 1228–1230, 2001.
87. Takasago T, Imagawa T, Furukawa K, Ogurusu T, and Shigekawa M. Regulation of the cardiac ryanodine receptor by protein kinase-dependent phosphorylation. *J Biochem (Tokyo)* 109: 163–170, 1991.
88. Thomas HM III, Carson RC, Fried ED, and Novitch RS. Inhibition of hypoxic pulmonary vasoconstriction by diphenyleneiodonium. *Biochem Pharmacol* 42: R9–R12, 1991.
89. Tsai BM, Wang M, Pitcher JM, Meldrum KK, and Meldrum DR. Hypoxic pulmonary vasoconstriction and pulmonary artery tissue cytokine expression are mediated by protein kinase C. *Am J Physiol Lung Cell Mol Physiol* 287: L1215–L1219, 2004.
90. Turner JL and Kozlowski RZ. Relationship between membrane potential, delayed rectifier K^+ currents and hypoxia in rat pulmonary arterial myocytes. *Exp Physiol* 82: 629–645, 1997.
91. Vadula MS, Kleinman JG, and Madden JA. Effect of hypoxia and norepinephrine on cytoplasmic free Ca^{2+} in pulmonary and cerebral arterial myocytes. *Am J Physiol* 265: L591–L597, 1993.
92. Vandier C, Delpech M, and Bonnet P. Spontaneous transient outward currents and delayed rectifier K^+ current: effects of hypoxia. *Am J Physiol* 275: L145–L154, 1998.
93. Wang J, Juhaszova M, Rubin LJ, and Yuan XJ. Hypoxia inhibits gene expression of voltage-gated K^+ channel alpha subunits in pulmonary artery smooth muscle cells. *J Clin Invest* 100: 2347–2353, 1997.
94. Wang J, Shimoda LA, Weigand L, Wang W, Sun D, and Sylvester JT. Acute hypoxia increases intracellular $[Ca^{2+}]$ in pulmonary arterial smooth muscle by enhancing capacitative Ca^{2+} entry. *Am J Physiol Lung Cell Mol Physiol* 288: L1059–L1069, 2005.
95. Wang J, Weigand L, Lu W, Sylvester JT, Semenza GL, and Shimoda LA. Hypoxia inducible factor 1 mediates hypoxia-induced TRPC expression and elevated intracellular Ca^{2+} in pulmonary arterial smooth muscle cells. *Circ Res* 98: 1528–1537, 2006.
96. Wang J, Weigand L, Wang W, Sylvester JT, and Shimoda LA. Chronic hypoxia inhibits Kv channel gene expression in rat distal pulmonary artery. *Am J Physiol Lung Cell Mol Physiol* 266: L1049–L1058, 2005.
97. Wang QS, Zheng YM, Dong L, Ho YS, Guo Z, and Wang YX. Role of mitochondrial reactive oxygen species in hypoxia-dependent increase in intracellular calcium in pulmonary artery myocytes. *Free Radic Biol Med* 42: 642–653, 2007.
98. Wang X, Tong M, Chinta S, Raj JU, and Gao Y. Hypoxia-induced reactive oxygen species downregulate ETB receptor-mediated contraction of rat pulmonary arteries. *Am J Physiol Lung Cell Mol Physiol* 290: L570–L578, 2006.
99. Wang YX, Zheng YM, Abdullaev II, and Kotlikoff MI. Metabolic inhibition with cyanide induces intracellular calcium release in pulmonary artery myocytes and *Xenopus* oocytes. *Am J Physiol Cell Physiol* 284: C378–C88, 2003.
100. Ward JP and Aaronson PI. Mechanisms of hypoxic pulmonary vasoconstriction: can anyone be right? *Respir Physiol* 115: 261–271, 1999.
101. Ward JP, Knock GA, Snetkov VA, and Aaronson PI. Protein kinases in vascular smooth muscle tone: role in the pulmonary vasculature and hypoxic pulmonary vasoconstriction. *Pharmacol Ther* 104: 207–231, 2004.
102. Waypa GB, Chandel NS, and Schumacker PT. Model for hypoxic pulmonary vasoconstriction involving mitochondrial oxygen sensing. *Circ Res* 88: 1259–1266, 2001.
103. Waypa GB, Guzy R, Mungai PT, Mack MM, Marks JD, Roe MW, and Schumacker PT. Increases in mitochondrial reactive oxygen species trigger hypoxia-induced calcium

responses in pulmonary artery smooth muscle cells. *Circ Res* 99: 970–978, 2006.
104. Waypa GB, Marks JD, Mack MM, Boriboun C, Mungai PT, and Schumacker PT. Mitochondrial reactive oxygen species trigger calcium increases during hypoxia in pulmonary arterial myocytes. *Circ Res* 91: 719–726, 2002.
105. Weigand LA, Wang J, Shimoda LA, Sham JS, and Sylvester JT. Inhibitors of capacitative calcium entry block hypoxic pulmonary artery vasoconstriction (HPV) in isolated rat lungs. *Am J Respir Crit Care Med* 167: A698, 2003.
106. Weir EK and Archer SL. Counterpoint: hypoxic pulmonary vasoconstriction is not mediated by increased production of reactive oxygen species. *J Appl Physiol* 101: 995–998, 2006.
107. Weir EK, Wyatt CN, Reeve HL, Huang J, Archer SL, and Peers C. Diphenyleneiodonium inhibits both potassium and calcium currents in isolated pulmonary artery smooth muscle cells. *J Appl Physiol* 76: 2611–2615, 1994.
108. Weissmann N, Dietrich A, Fuchs B, Kalwa H, Ay M, Dumitrascu R, Olschewski A, Storch U, Schnitzler M, Ghofrani HA, Schermuly RT, Pinkenburg O, Seeger W, Grimminger F, and Gudermann T. Classical transient receptor potential channel 6 (TRPC6) is essential for hypoxic pulmonary vasoconstriction and alveolar gas exchange. *Proc Natl Acad Sci USA* 103: 19093–19098, 2006.
109. Weissmann N, Ebert N, Ahrens M, Ghofrani HA, Schermuly RT, Hanze J, Fink L, Rose F, Conzen J, Seeger W, and Grimminger F. Effects of mitochondrial inhibitors and uncouplers on hypoxic vasoconstriction in rabbit lungs. *Am J Respir Cell Mol Biol* 29: 721–732, 2003.
110. Weissmann N, Tadic A, Hanze J, Rose F, Winterhalder S, Nollen M, Schermuly RT, Ghofrani HA, Seeger W, and Grimminger F. Hypoxic vasoconstriction in intact lungs: a role for NADPH oxidase- derived H_2O_2? *Am J Physiol Lung Cell Mol Physiol* 279: L683–L690, 2000.
111. Weissmann N, Voswinckel R, Hardebusch T, Rosseau S, Ghofrani HA, Schermuly R, Seeger W, and Grimminger F. Evidence for a role of protein kinase C in hypoxic pulmonary vasoconstriction. *Am J Physiol* 276: L90–L95, 1999.
112. Weissmann N, Zeller S, Schafer RU, Turowski C, Ay M, Quanz K, Ghofrani HA, Schermuly RT, Fink L, Seeger W, and Grimminger F. Impact of mitochondria and NADPH oxidases on acute and sustained hypoxic pulmonary vasoconstriction. *Am J Respir Cell Mol Biol* 34: 505–513, 2006.
113. Wilhelm J and Herget J. Role of ion fluxes in hydrogen peroxide pulmonary vasoconstriction. *Physiol Res* 44: 31–37, 1995.
114. Wu W, Platoshyn O, Firth AL, and Yuan JX. Hypoxia divergently regulates production of reactive oxygen species in human pulmonary and coronary artery smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 293: L952–L959, 2007.
115. Xin HB, Senbonmatsu T, Cheng DS, Wang YX, Copello JA, Ji GJ, Collier ML, Deng KY, Jeyakumar LH, Magnuson MA, Inagami T, Kotlikoff MI, and Fleischer S. Oestrogen protects FKBP12.6 null mice from cardiac hypertrophy. *Nature* 416: 334–337, 2002.
116. Yamaguchi K, Asano K, Mori M, Takasugi T, Fujita H, Suzuki Y, and Kawashiro T. Constriction and dilatation of pulmonary arterial ring by hydrogen peroxide: importance of prostanoids. *Adv Exp Med Biol* 361: 457–463, 1994.
117. Yang XR, Lin MJ, Yip KP, Jeyakumar LH, Fleischer S, Leung GP, and Sham JS. Multiple ryanodine receptor subtypes and heterogeneous ryanodine receptor-gated Ca^{2+} stores in pulmonary arterial smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 289: L338–L348, 2005.
118. Zhang F, Carson RC, Zhang H, Gibson G, and Thomas HM III. Pulmonary artery smooth muscle cell $[Ca^{2+}]_i$ and contraction: responses to diphenyleneiodonium and hypoxia. *Am J Physiol* 273: L603–L611, 1997.
119. Zhang YC, Ni W, Zhang ZK, and Xu YJ. The effect of protein kinase C on voltage-gated potassium channel in pulmonary artery smooth muscle cells from rats exposed to chronic hypoxia. *Chin Med J (Engl)* 117: 19–23, 2004.
120. Zheng YM, Mei QB, Wang QS, Abdullaev I, Lai FA, Xin HB, Kotlikoff MI, and Wang YX. Role of FKBP12.6 in hypoxia- and norepinephrine-induced Ca^{2+} release and contraction in pulmonary artery myocytes. *Cell Calcium* 35: 345–355, 2004.
121. Zheng YM, Wang QS, Liu QH, Rathore R, Yadav V, and Wang YX. Heterogeneous gene expression and functional activity of ryanodine receptors in resistance and conduit pulmonary as well as mesenteric artery smooth muscle cells. *J Vasc Res* 45: 469–479, 2008.
122. Zheng YM, Wang QS, Rathore R, Zhang WH, Mazurkiewicz JE, Sorrentino V, Singer HA, Kotlikoff MI, and Wang YX. Type-3 ryanodine receptors mediate hypoxia-, but not neurotransmitter-induced calcium release and contraction in pulmonary artery smooth muscle cells. *J Gen Physiol* 125: 427–440, 2005.

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Abbreviations Used

$[Ca^{2+}]_i$ = intracellular Ca^{2+} concentration
Ca_V = voltage-dependent Ca^{2+}
ETC = electron-transport chain
FKBP12.6 = FK506-binding protein with a molecular mass of 12.6kDa
Gpx1 = glutathione peroxidase-1
H_2DCF = dichlorodihydrofluorescein
H_2O_2 = hydrogen peroxide
K_V = voltage-dependent K^+
Nox = NADPH oxidase
O_2^- = superoxide anion
PASMC = pulmonary artery smooth muscle cell
PKC = protein kinase C
$[ROS]_i$ = intracellular reactive oxygen species concentration
RyR = ryanodine receptor
SOC = store-operated Ca^{2+}
SR = sarcoplasmic reticulum
TRPC = canonic transient receptor potential

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2. Jiwei Zhang , Juan Zhou , Lei Cai , Yankai Lu , Tao Wang , Liping Zhu , Qinghua Hu . 2012. Extracellular Calcium-Sensing Receptor Is Critical in Hypoxic Pulmonary Vasoconstriction. *Antioxidants & Redox Signaling* **17**:3, 471-484. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
3. Eberhard Schulz , Philip Wenzel , Thomas Münzel , Andreas Daiber . Mitochondrial Redox Signaling: Interaction of Mitochondrial Reactive Oxygen Species with Other Sources of Oxidative Stress. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
4. Yi-Hong Liu , Ming Lu , Li-Fang Hu , Peter T.-H. Wong , George D. Webb , Jin-Song Bian . 2012. Hydrogen Sulfide in the Mammalian Cardiovascular System. *Antioxidants & Redox Signaling* **17**:1, 141-185. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
5. Li Long Pan , Xin Hua Liu , Qi Hai Gong , He Bei Yang , Yi Zhun Zhu . 2012. Role of Cystathionine #-Lyase/Hydrogen Sulfide Pathway in Cardiovascular Disease: A Novel Therapeutic Strategy?. *Antioxidants & Redox Signaling* **17**:1, 106-118. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
6. Benjamin Lee Predmore , David Joseph Lefer , Gabriel Gojon . 2012. Hydrogen Sulfide in Biochemistry and Medicine. *Antioxidants & Redox Signaling* **17**:1, 119-140. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
7. Jie Gao, Yi-qun Tang, De-zai Dai, Yu-si Cheng, Guo-lin Zhang, Can Zhang, Yin Dai. 2012. Raisanberine protected pulmonary arterial rings and cardiac myocytes of rats against hypoxia injury by suppressing NADPH oxidase and calcium influx. *Acta Pharmacologica Sinica* **33**:5, 625-634. [CrossRef]
8. Marta R. Gorowiec, Lee A. Borthwick, Sean M. Parker, John A. Kirby, Gabriele C. Saretzki, Andrew J. Fisher. 2012. Free Radical Generation Induces Epithelial-to-Mesenchymal Transition in Lung Epithelium via a TGF-#1-dependent Mechanism. *Free Radical Biology and Medicine* . [CrossRef]
9. Si Jin , Fan Zhou , Foad Katirai , Pin-Lan Li . 2011. Lipid Raft Redox Signaling: Molecular Mechanisms in Health and Disease. *Antioxidants & Redox Signaling* **15**:4, 1043-1083. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
10. Amit S. Korde, Vishal R. Yadav, Yun-Min Zheng, Yong-Xiao Wang. 2011. Primary role of mitochondrial Rieske iron–sulfur protein in hypoxic ROS production in pulmonary artery myocytes. *Free Radical Biology and Medicine* **50**:8, 945-952. [CrossRef]
11. Bo Liao , Yun-Min Zheng , Vishal R. Yadav , Amit S. Korde , Yong-Xiao Wang . 2011. Hypoxia Induces Intracellular Ca2+ Release by Causing Reactive Oxygen Species-Mediated Dissociation of FK506-Binding Protein 12.6 from Ryanodine Receptor 2 in Pulmonary Artery Myocytes. *Antioxidants & Redox Signaling* **14**:1, 37-47. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
12. Yong-Xiao Wang, Yun-Min Zheng. 2010. Role of ROS signaling in differential hypoxic Ca2+ and contractile responses in pulmonary and systemic vascular smooth muscle cells#. *Respiratory Physiology & Neurobiology* **174**:3, 192-200. [CrossRef]
13. C. Gonzalez, M.T. Agapito, A. Rocher, A. Gomez-Niño, R. Rigual, J. Castañeda, S.V. Conde, A. Obeso. 2010. A revisit to O2 sensing and transduction in the carotid body chemoreceptors in the context of reactive oxygen species biology#. *Respiratory Physiology & Neurobiology* **174**:3, 317-330. [CrossRef]
14. Francisco Perez-Vizcaino, Angel Cogolludo, Laura Moreno. 2010. Reactive oxygen species signaling in pulmonary vascular smooth muscle#. *Respiratory Physiology & Neurobiology* **174**:3, 212-220. [CrossRef]
15. Navdeep S. Chandel. 2010. Mitochondrial complex III: An essential component of universal oxygen sensing machinery?#. *Respiratory Physiology & Neurobiology* **174**:3, 175-181. [CrossRef]
16. Rui Wang . 2010. Hydrogen Sulfide: The Third Gasotransmitter in Biology and Medicine. *Antioxidants & Redox Signaling* **12**:9, 1061-1064. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
17. David Jourd'heuil . 2010. Redox Control of Vascular Smooth Muscle Function. *Antioxidants & Redox Signaling* **12**:5, 579-581. [Citation] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
18. Tomoka MORITA, Hideyuki YAMAWAKI, Muneyoshi OKADA, Yukio HARA. 2010. Contractile Characteristics of Rat Mesenteric Artery after Organ Culture. *Journal of Veterinary Medical Science* **72**:12, 1621-1627. [CrossRef]