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Forum Review

Oxidant and Redox Signaling in Vascular Oxygen Sensing: Implications for Systemic and Pulmonary Hypertension

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ABSTRACT

It has been well known for >100 years that systemic blood vessels dilate in response to decreases in oxygen tension (hypoxia; low Po_2), and this response appears to be critical to supply blood to the stressed organ. Conversely, pulmonary vessels constrict to a decrease in alveolar Po_2 to maintain a balance in the ventilation-to-perfusion ratio. Currently, although little question exists that the Po_2 affects vascular reactivity and vascular smooth muscle cells (VSMCs) act as oxygen sensors, the molecular mechanisms involved in modulating the vascular reactivity are still not clearly understood. Many laboratories, including ours, have suggested that the intracellular calcium concentration ($[Ca^{2+}]_i$), which regulates vasomotor function, is controlled by free radicals and redox signaling, including NAD(P)H and glutathione (GSH) redox. In this review article, therefore, we discuss the implications of redox and oxidant alterations seen in pulmonary and systemic hypertension, and how key targets that control $[Ca^{2+}]_i$, such as ion channels, Ca^{2+} release from internal stores and uptake by the sarcoplasmic reticulum, and the Ca^{2+} sensitivity to the myofilaments, are regulated by changes in intracellular redox and oxidants associated with vascular Po_2 sensing in physiologic or pathophysiologic conditions. *Antioxid. Redox Signal*. 10, 1137–1152.

"Only when free oxygen appeared in the atmosphere—some billion years ago—did the higher development of life set in, to produce the plant and animal kingdoms from the fermenting, undifferentiated single cells." Otto Warburg, 1931

Molecular oxygen is essence of life, and O_2 sensing is a fundamental biologic process that allows an organism to adapt to physiologic situations. Cellular responses to changes in partial oxygen tension (Po_2) are acute. In 1964, Guyton and colleagues (59) showed that autoregulation of the femoral artery in the hindlimb of a dog is caused by decreasing Po_2 in vascular smooth muscle, and later it was proposed that a Po_2 sensor was present in vascular smooth muscle (29). Now, it is well established that vascular smooth muscle cells (VSMCs) are O_2 sensors containing the fast-responsive elements, which either dilate or contract the blood vessel to adjust blood flow to an organ, depending on the O_2 demand. Pulmonary arteries constrict to alveolar hypoxia (low Po_2) to maintain the ventilation-to-perfusion ratio. In contrast, systemic blood vessels dilate to low Po_2, and this response appears to be critical to supply blood to the stressed organ (59). Nevertheless, although little question exists that Po_2 affects vascular reactivity, the nature of O_2 sensor molecules that regulate vascular smooth muscle function and the mechanisms of interaction between the sensors and the effectors that are ultimately involved in modulating the vascular reactivity are still not clearly understood.

The latest evidence suggests that Po_2 modulates the cell-signaling pathways involved in excitation–contraction coupling, including mechanisms in which the intracellular calcium concentration ($[Ca^{2+}]_i$) is the regulated parameter, for example, by oxygen-dependent or ATP-dependent changes in Ca^{2+} permeability (43, 87). Interestingly, both $[Ca^{2+}]_i$ -dependent and -independent (*i.e.*, by decreasing Ca^{2+} sensitivity to the contractile apparatus) mechanisms are evoked in relaxing porcine coronary artery to different levels of hypoxia (137). It has been determined by whole-cell patch-clamp studies of dispersed VSMCs from rabbit and porcine systemic arteries, as well as the main pulmonary artery, that activation of voltage-gated Ca^{2+} channels is sensitive to O_2, and a reduction of Po_2 rapidly and reversibly inhibits Ca^{2+} currents (43, 138, 152). Conversely, voltage-gated Ca^{2+} channels from small pulmonary

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artery smooth muscle cells are activated by lowering of P_{O_2} (42, 152), and presumably this contributes to hypoxic pulmonary vasoconstriction and, with prolonged exposure to hypoxia, to the development of pulmonary hypertension. These findings, therefore, suggest that ion channel function, $[Ca^{2+}]_i$, and Ca^{2+} sensitivity to the contractile apparatus in the VSMCs are regulated by changes in P_{O_2}.

In the past, studies have proposed that mitochondria, NAD(P)H oxidase, and K^+ channels in VSMCs serve as P_{O_2} sensors and regulators of vascular function (163). The release of an endothelium-derived hyperpolarizing factor and loss of ATP or elevation of adenosine by hypoxia have been implicated in eliciting arterial dilation through opening calcium-activated (K_{Ca}) and ATP-dependent (K_{ATP}) K^+ channels, respectively (75). Studies have suggested that K_{Ca} channels located in proximity to sarco(endo)plasmic recticulum (SR) Ca^{2+} stores are activated by Ca^{2+} sparks to promote hyperpolarization and relaxation of vascular smooth muscle (167). In pulmonary artery smooth muscle, inactivation of voltage-dependent K^+ ($K_{v1.5}$) channels has been implicated to mediate hypoxic pulmonary vasoconstriction and pulmonary hypertension (8, 96). Thus, hypoxia seems to evoke the relaxation of coronary arteries and constriction of pulmonary arteries from a variety of species, through mechanisms often involving the opening or closing of K^+ channels (31, 49, 96).

Several theories explain how ion channels and vascular smooth muscle function are regulated by hypoxia, as shown in schematic illustrations in model figures for pulmonary arteries (Fig. 1) and systemic arteries (Fig. 2). One hypothesis suggests that oxidants, including H_2O_2, derived from mitochondria, activate $K_{v1.5}$ channels. During hypoxia, it has been reported that the production of mitochondrial H_2O_2 is shut off, resulting in inactivation of $K_{v1.5}$ channels and membrane depolarization, associated with pulmonary artery vasoconstriction (8, 96). Another hypothesis is that H_2O_2 derived from NAD(P)H oxidases (Nox) relaxes bovine pulmonary artery by increasing intracellular cGMP, and under hypoxia, a downregulation of H_2O_2 production decreases cGMP, and this elicits pulmonary artery constriction (23, 51, 103). Conversely, Marshall *et al*. (91) proposed that NAD(P)H oxidase–derived O_2^- is increased by hypoxia in pulmonary arteries, and inhibition of NAD(P)H oxidase by diphenyliodonium decreases hypoxic pulmonary vasoconstriction. Some hypotheses suggest that hypoxia promotes Ca^{2+} release in pulmonary arteries as a result of increasing mitochondria-derived H_2O_2 (157, 159). Additionally, it has been suggested that changes in the intracellular redox potential in response to alterations in P_{O_2} may be involved in regulating ion channel function, because $NADP^+$/NADPH, NAD^+/NADH, and GSSG/GSH regulate voltage- and Ca^{2+}-dependent K^+ channels in pulmonary arteries (81, 117, 118, 163). Recently, we identified that pentose phosphate pathway (PPP)-derived NADPH redox regulates voltage-dependent K^+ and L-type Ca^{2+} channel activity (52, 55). Inhibition of the PPP by 6-aminonicotinamide and epiandrosterone almost completely relaxes rat aorta and pulmonary artery preconstricted by depolarization and receptor-activated mechanisms, and abolishes hypoxic pulmonary vasoconstriction (52, 53) and pulmonary hypertension (15, 114). Although K^+ channel blockers reduced the dilation elicited by PPP inhibition, the majority of the relaxation response is not affected by K^+ channel blockade (52). Instead, we found that preconstricted bovine coronary arteries are dilated in response to inhibition of the

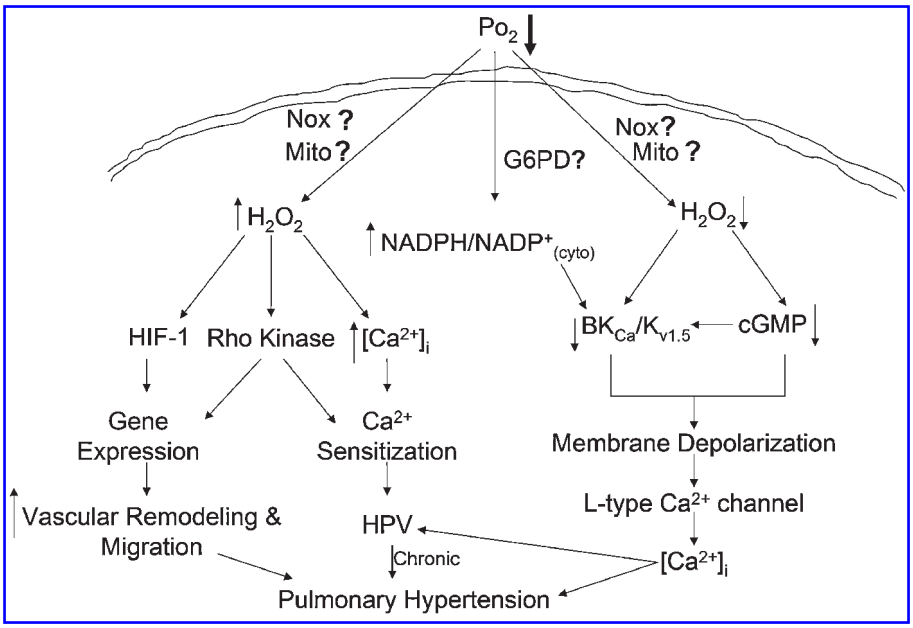

FIG. 1. Two major hypotheses are proposed to explain the cause of hypoxic pulmonary vasoconstriction (HPV) and pulmonary hypertension (PH). One hypothesis is that acute hypoxia inhibits hydrogen peroxide (H_2O_2) production from NADPH oxidases (Nox) and/or mitochondria (Mito). Decreases in peroxide inactivate calcium-dependent and voltage-gated K^+ channels (BK_{Ca} and $K_{v1.5}$, respectively), resulting in membrane depolarization and opening of L-type Ca^{2+} channels. This increases intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), which evokes HPV, and prolonged HPV promotes PH. Another hypothesis suggests that Mito- and Nox-derived H_2O_2 is elevated in pulmonary artery smooth muscle by prolonged/persistent hypoxia, and this elevates $[Ca^{2+}]_i$, and activates HIF-1 and Rho kinase. These changes enhance Ca^{2+} sensitization and evoke HPV, and eventually trigger gene expression, which induces vascular remodeling and growth. The modulation of metabolism by glucose-6-phosphate dehydrogenase (G6PD) by hypoxia also increases the cytosolic NADPH-to-$NADP^+_{(cyto)}$ ratio, and this either inactivates K_v channels and thereby depolarizes the membrane potential, which activates L-type Ca^{2+} currents, or it directly activates L-type Ca^{2+} channels, and the $[Ca^{2+}]_i$ increases to initiate HPV.

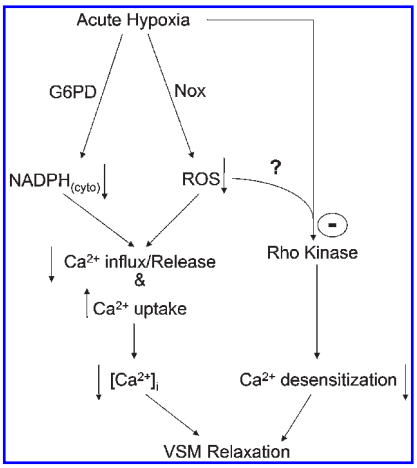

FIG. 2. In systemic arteries, acute hypoxia inhibits glucose-6-phosphate dehydrogenase (G6PD) and NADPH oxidases (Nox), which reduces Ca^{2+} influx and release, or accelerates Ca^{2+} uptake by sarco(endo)plasmic reticulum and decreases $[Ca^{2+}]_i$. Additionally, Rho kinase inactivation by acute hypoxia can induce Ca^{2+} desensitization. These processes, therefore, mediate hypoxic relaxation.

PPP, and the relaxation is caused by a decrease in $[Ca^{2+}]_i$ resulting from attenuated Ca^{2+} influx and release, which appears to be controlled by NADPH and glutathione (GSH) redox (50). These observations are supported by another experiment in which epiandrosterone, a PPP inhibitor, directly reduces myocardial contractility by decreasing the open probability and the inactivation time of L-type Ca^{2+} channels (55). Furthermore, studies in bovine coronary arteries suggest that decreased Ca^{2+} influx and release, which appear to be mediated by inhibition of an O_2-sensitive aspect of the PPP controlling changes in NADPH/GSH redox, are involved in mediating relaxation of the systemic blood vessels in response to decrease in P_{O_2} (56). Interestingly, this relaxation to hypoxia appears to function in a manner that is not dependent on the function of K^+ channels. In human internal mammary and radial arteries, accelerated Ca^{2+} uptake by sarco(endo)plasmic recticulum ATPase (SERCA) appears as a major mechanism involved in mediating dilation of these systemic blood vessels in response to hypoxia (58). Although it is evident from these studies that Ca^{2+} signaling is altered by changes in P_{O_2}, the precise intracellular signaling systems involved in O_2 sensing are not yet clearly understood. Regulation of VSMC contraction–relaxation function is a complex process involving multiple intracellular signaling pathways. Low P_{O_2} and free radicals are well known to regulate VSMC function. Therefore, goal of this article is to review broadly the "fundamental" intracellular signaling pathways activated by the changes in oxidants and redox potential leading to vascular contraction–relaxation and to discuss the current opinion about the sensor, effectors, and the role of oxidant and redox signaling in mediating pulmonary artery hypoxic vasoconstriction and systemic artery hypoxic vasodilation. An additional focus is considering how the function of vascular P_{O_2} sensing mechanisms are potentially altered in pulmonary and systemic hypertension.

POSSIBLE O_2 SENSORS AND MECHANISM OF OXYGEN SENSING IN VSMCS

The carotid bodies or carotid glomus is a small cluster of chemoreceptors and supporting cells located near the bifurcation of the carotid artery. The type I glomus cells in carotid bodies are primary O_2 sensors, because they measure changes in the composition of arterial blood flowing through them, including the partial pressures of oxygen and carbon dioxide, and regulate ventilation rate. In addition, because isolated arteries respond directly to hypoxia by either dilating or constricting, VSMCs are also considered to contain O_2 sensors that are involved in sensing acute changes in P_{O_2}. This phenomenon is potentially vital to sensing changes in local P_{O_2} in organs such as in the heart or in the lungs evoked by conditions such as coronary artery vasospasm and pulmonary ventilation-to-perfusion mismatch due to obstruction, respectively, which acutely regulate blood flow in these organs. For example, the blood flow is generally matched to the metabolic needs of the working heart by the resistance coronary arteries, because the smooth muscle in the resistance coronary arteries senses changes in the demand-to-supply ratio, and this increase in blood flow caused by hypoxia also appears to dilate the conduit-size epicardial coronary arteries. Similarly, in the lungs, the precapillary smooth muscle layer of the resistance pulmonary arteries, located at the acinus entrance, has been identified to sense and respond to changes in P_{O_2} in a manner that maintains a balance in ventilation-to-perfusion ratio (32, 63, 76, 140); whereas, in skeletal muscle ATP, adenosine and lactic acid are released in response to lowering of P_{O_2} and these metabolites also potentially act on smooth muscle cells in arterioles to autoregulate blood flow to the organ (45, 141). For this reason, the metabolic enzymes are considered to be potential candidates for P_{O_2} sensing, and the substrate requirement for the enzymes that metabolize O_2 is one of the most fundamental mechanisms of P_{O_2} sensing at the cellular level. Although it is now clear that VSMCs are the sensors and effectors, an ideal protein that detects changes in P_{O_2} by binding to O_2 in a manner that is directly linked to the control of cellular functions is yet to be identified in vascular tissue. Instead, several types of enzymes appear to sense changes in P_{O_2}, and they include complex I and III of the mitochondrial respiratory chain, involved in generating superoxide anion by transferring one electron to molecular O_2; hydroxylases that modify protein groups (*e.g.*, proline in hypoxia-inducible factor-1); and oxidoreductases that generate reactive oxygen species (169). The aforementioned proteins are conserved in evolution, and they are functionally important in an organism. Although these proteins have an O_2-sensing PAS domain and their role in O_2 sensing has been recognized, the molecular mechanisms of O_2 sensing by these proteins are poorly understood; therefore, the focus of the current section is to discuss the functional aspects of these proteins as O_2 sensors.

Mitochondria

In 1948, Lehninger (34) showed that in the animal cell, the mitochondrion is the sole site for oxidative phosphorylation, the tricarboxylic acid cycle, and fatty acid oxidation. This led the

early studies to consider electron transport and oxidative phosphorylation in mitochondria as being critical for the depression of arterial smooth muscle contraction evoked by reducing the P_{O_2} (34); however, subsequent studies provided evidence that suggested that the mitochondrial energy metabolism required for the generation of force was not involved in P_{O_2} sensing (30, 34). In 1981, Round and McMurtry (131) found that the mitochondrial respiratory chain inhibitors (rotenone, antimycin A, azide, and cyanide), as well as a mitochondrial uncoupling agent (dinitrophenol), increased vascular pressure under normoxic conditions and inhibited subsequent hypoxic pulmonary vasoconstriction in *ex vivo* blood-perfused rat lungs. Later, Archer and Weir (6) demonstrated that hypoxia and the respiratory chain inhibitors, rotenone and antimycin A, decreased oxidant production and increased pulmonary artery pressure. These studies led to the reconsideration of complex I and III of mitochondria as a site of P_{O_2} sensing in pulmonary arterial smooth muscle. Subsequently, Archer and co-workers (96) reported that these agents mimicked hypoxic pulmonary vasoconstriction in isolated pulmonary arteries (PAs) and in pulmonary artery smooth muscle cells (PASMCs). In contrast, mitochondrial inhibitors increased oxidant generation and dilated renal arteries (96). They attributed the difference in the response of pulmonary as compared with renal artery to the lower respiration rates in lung mitochondria, lower complex I and III levels, and a more hyperpolarized mitochondrial membrane potential ($\Delta\Psi_m$). Although it is now well accepted that mitochondrial complex I and III may play a role in P_{O_2} sensing in rat PASMCs, it is noteworthy that the role for mitochondrial complexes in P_{O_2} sensing in other species is poorly understood. Also, whether these complexes initiate signaling in rat PASMCs by decreasing or increasing ROS production under hypoxia is controversial, and more important, how these complexes sense change in P_{O_2} is not well understood. These complexes could detect changes in P_{O_2} through multiple mechanisms, and they include (a) a decrease in O_2 gradient across the cell and lower diffusion of O_2 to mitochondrion could decrease in levels of NADH, an electron donor, because of a shutdown of aerobic glycolysis (79, 80); (b) a change in $\Delta\Psi_m$ after modulation of mitochondrial K^+ channels by low P_{O_2} (5); and (c) under severe or chronic hypoxic conditions, ATPase inhibitor subunit, IF1, could fail to prevent hydrolysis of ATP and impairment of proton pump function (132). All of these conditions are known to uncouple oxidative phosphorylation from the electron transport chain (ETC) in a manner that generates ROS from mitochondria and potentially plays a significant role in modulating vascular smooth muscle function.

Glucose-6-phosphate dehydrogenase

In most aerobic organisms, oxidative phosphorylation is heavily dependent on glycolysis, and hypoxia appears to modulate glucose uptake as well as glycolysis in opposite directions in pulmonary and systemic (mesenteric) arteries. For instance, glucose uptake is increased and glycolysis is severely downregulated in pulmonary arteries by hypoxia, whereas glucose uptake and glycolysis are increased by 40–50% in mesenteric arteries under hypoxia (80). Recently, we reported that the levels of glucose-6-phosphate, which is a substrate for the glycolytic as well as the pentose shunt pathways, are increased in pulmonary arteries by decreasing P_{O_2} from 140 to 40 torr (53), as opposed to hypoxia causing a decrease in its levels in coronary arteries (CAs) (56). We also demonstrated in these studies that G6PD activity, as determined by increase in the NADPH-to-$NADP^+$ ratio and NADPH absolute values, is increased in lungs (52) and pulmonary arteries (52, 53). These changes are in striking contrast to G6PD activity and NADPH-to-$NADP^+$ ratios being decreased in coronary arteries (56). Therefore, it is reasonable to speculate that changes in substrate availability and NADPH-to-$NADP^+$ ratios by hypoxia is presumably a primary cause of increased or decreased G6PD activity in pulmonary and coronary arteries, respectively. Interestingly, when the PPP was discovered by Otto Warburg in 1930s, he described in his first of a series of classic articles that G6PD, a rate-limiting enzyme in PPP activity, was regulated by NADPH metabolism in an O_2-dependent manner (154). We now also know that NADPH redox controls ion channel activity (52, 55) and guanylate cyclase activity (54), which regulate VSMC function. Therefore, we propose that G6PD has an important role in P_{O_2} sensing by the virtue of its capabilities to sense and control metabolic changes and NADPH redox. More important, G6PD activity is also acutely regulated by changes in intracellular GSH and H_2O_2. It is further intriguing to know that the G6PD promoter is activated by an elevation in H_2O_2 (78), which appears to increase in smooth muscle when it is exposed to persistent hypoxic conditions (133). The expression of G6PD is upregulated by hypoxia-inducible factor-1 (HIF-1) in PC12 cells exposed to chronic hypoxia (46). In this regard, it is noteworthy that G6PD expression is also found to be upregulated in the lungs of rats exposed to the Denver atmosphere (Gupte and Oka, 2005; unpublished observation) and in erythrocytes from the Aymaras of the high Andean Plateau (9). Even though no reports suggest elevated G6PD expression in lungs of people living at high altitudes (10,000 to 15,000 feet), such as Himalayan ranges, studies have shown that HIF-1, which upregulates G6PD gene expression, is overexpressed in these natives because of adaptation (44, 149, 170). Therefore, it is not unreasonable to extrapolate that G6PD expression could also be altered in lungs of natives living at high-altitude regions of the world. Qualities associated with G6PD to sense acute changes in P_{O_2} by detecting modulation in metabolic substrates, coenzymes, or ROS generation, as well as chronic changes in P_{O_2} sensed through increased G6PD gene expression that is regulated through activation of HIF-1 (46), therefore, makes G6PD an attractive and potentially important component of O_2 sensor mechanisms.

NAD(P)H oxidase

The role of NADPH oxidases as a generator of superoxide anion (O_2^-) and H_2O_2 during the respiratory burst associated with phagocytosis in neutrophils has been well recognized since the 1960s (71, 119). These enzymes are thought to have Nox subunits that contain a flavin electron-transfer system (b_{558}-type flavocytochrome) and generate O_2^- by transferring an electron from the PPP-derived NADPH to molecular O_2 (70). The reduced (Fe^{2+}) form of flavocytochrome b_{558} appears to react directly with oxygen at a very rapid rate (10^{-7} M/sec at 10°C) (70). In mid 1980s, the neutrophilic isoform of NADPH oxidase, $gp91^{phox}$, also known as Nox-2, was discovered in bovine

endothelium and vascular smooth muscle (102, 104) and in fibroblasts from the adventitia of rabbit aorta (115). Currently, variant forms of Nox-2, Nox-1, and Nox-4, are also expressed in the rat and bovine blood vessels and are thought to be a major source of O_2^- generation (51, 89). Additionally, another variant of Nox-2 protein, Nox-5, activated by changes in intracellular Ca^{2+} concentration, has been detected in human tissue including arterial smooth muscle (13, 67). These oxidases are anchored to the plasma membrane by $p22^{phox}$, and under basal conditions, they are constitutively active and produce low levels of ROS (51). Stimulation of blood vessels through pathways such as the protein kinase C or Src kinase promotes phosphorylation of $p47^{phox}$, a cytosolic subunit of NADPH oxidase, and a complex formation of $p47^{phox}$ with $p67^{phox}$. This complex and rac1 translocate to the plasma-membrane sites containing Nox oxidases and accelerating the flow of e^- from NADPH into a flavin-linked cytochrome b_{558} that consequently reduces molecular oxygen into O_2^- (89, 169). Activation of phosphatidylinositol 3-kinase (PI3K) and phospholipase D produces 3-phosphorylated phosphatidylintositols (PtdInsP) and phosphatidic acid, respectively, providing lipids to which the $p47^{phox}$ (and $p40^{phox}$) PX domains bind, associated with a facilitation of O_2^- production (89). Decreases in the direct binding of O_2 to flavocytochrome b_{558} needed for O_2^- production and inhibition of pathways promoting complex formation of Nox and cytosolic subunits by reduction in P_{O_2} may be some of the mechanisms of O_2 sensing by Nox. Therefore, these oxidases are considered to be potential O_2 sensors. Two views exist about NADPH oxidases–derived ROS generation under hypoxia; one opinion suggests that ROS are decreased, and the second view is that ROS are increased by hypoxia. NADPH oxidase in carotid bodies was initially recognized as a P_{O_2} sensor (2), and based on these studies, evidence was provided that diphenyleneiodinium, a nonselective NADPH oxidase inhibitor, decreased hypoxic pulmonary vasoconstriction in isolated-perfused lungs (144). Observations by Mohazzab-H and Wolin (101, 103, 104) that NADPH oxidases were the major source of O_2^- production in bovine pulmonary arteries, and the modulation of ROS production by P_{O_2} resulted in the proposal that it could function as a key vascular P_{O_2} sensor. These authors provided evidence that hypoxia decreased ROS production and concomitantly relaxed pulmonary arteries. Conversely, a study by Marshall *et al*. (58) suggested that enhanced generation of O_2^- from large and resistance bovine arterial smooth muscle was inhibited by diphenyleneiodonium, but not by myxothiazol, a mitochondrial complex III inhibitor, under hypoxia. They also reported that diphenyleneiodonium decreased contraction to hypoxia in cat pulmonary arteries without increasing force.

Subsequently, Weissmann and colleagues (166) showed a decrease in the contraction to hypoxia by a Nox oxidase inhibitor [4-(2-aminoethyl) benzenesulfonyl fluoride], which occurred without increases in baseline perfusion pressure or agonist-induced contraction under normoxia in saline-perfused rabbit lungs. These observations led to the suggestion that an increased Nox-derived ROS under hypoxia plays a role in modulating hypoxic pulmonary vasoconstriction. However, observations that pulmonary vessels from mice lacking $gp91^{phox}$ (Nox-2) show normal contractile responses to hypoxia, associated with decreased detection of superoxide (7), raises questions about a key role for Nox-2–derived ROS in the hypoxia-elicited contractile response. Similarly, apocynin appears to inhibit basal Nox-2 activity in bovine pulmonary arteries (51) and does not appear to attenuate hypoxia-elicited increases (in Nox activity) in cultured rat pulmonary arterial smooth muscle cells (157). It should be noted that apocynin does not mimic the effects of hypoxia in rat lungs (157). Nonetheless, acute hypoxic pulmonary vasoconstriction is reduced by 25%, but sustained hypoxic pulmonary vasoconstriction is unaffected in $p47^{phox}$-deficient mice, suggesting that NADPH oxidase may, at least partly, function as a P_{O_2} sensor (165).

Furthermore, recent studies have detected Nox-4 (in addition to Nox-2) in pulmonary arteries that contract to hypoxia (51), and it is interesting to note that Nox-4, initially described as a renal Nox (renox), was suggested to be a P_{O_2} sensor involved in controlling the production of erythropoietin (47). Therefore, it seems reasonable to speculate that NADPH oxidases other than Nox-2, like Nox-4 or Nox-5, may play a role in P_{O_2} sensing in VSMCs, and the plausibility of this concept remains to be investigated.

Hypoxia-inducible factor-1

HIF-1 is a transcription factor (TF) involved in mediating the physiologic response to hypoxia, and its importance is emphasized by the fact that this TF is found in a wide variety of different multicellular organisms, ranging from nematodes to mammals (61). HIF-1 is a PAS domain containing heterodimeric TF that is composed of α- and β-subunits. The α-subunit senses O_2 changes; in the presence of O_2, the α-subunit is hydroxylated at Pro-564 (and/or Pro-402) by specific prolyl hydroxylases in a manner that requires Fe^{2+}, 2-oxoglutarate, and molecular O_2 (19, 61). Once Pro-564 is hydroxylated, it interacts with the von Hippel–Lindau protein, which is part of the E3 ubiquitin ligase complex, and once ubiquitinated, HIF-1α is rapidly degraded by the ubiquitin–proteasome pathway (61). Under hypoxia, degradation of HIF-1α is slowed, as a result of which HIF-1α accumulates in the cell and induces transcription of genes. By current thinking, hypoxia is "sensed" by hydroxylases that permit HIF-1α to complex with HIF-1β to form a transcriptional activator that drives expression of hypoxia-sensitive genes (such as erythropoeitin) under hypoxic conditions. In altitude-adapted Andean natives, hypoxia sensing is changed, and this is a cause of alterations in target gene expression (64). Although HIF-1 does not seem to regulate oxidant generation or redox signaling, conflicting evidence suggests it is oxidant and redox sensitive (3). Therefore, we speculate that oxidants produced by O_2 sensors, such as mitochondria and NAD(P)H oxidases, in response to acute changes in P_{O_2}, could subsequently lead to the activation of HIF-1, thereby leading to the expression of early-response genes. Transcription of early-response genes is known to modulate cardiovascular function (25) and participate in vascular remodeling in the lung (150).

Chronic hypoxia could affect endothelial function; therefore, one should also consider involvement of endothelium-derived factors and hence the role of oxygenase enzymes that generate autocoids on O_2 sensing. However, conflicting evidence regards whether prostaglandins and NO participate in mediating systemic and pulmonary arterial responses elicited by changes in P_{O_2}. Often it appears that these mediators modulate vascular re-

sponses to changes in P_{O_2}. Therefore, the role of these enzymes in P_{O_2} sensing is not discussed in detail in this review.

ROLE OF POTENTIAL VSMC EFFECTORS IN CONTROLLING VASCULAR FUNCTION AND THEIR REGULATION BY OXIDANT AND REDOX IN VSM

In this section, we discuss the nature of potential effectors that are currently known to be regulated by changes in P_{O_2}. Generally, the effectors are protein processors in VSMCs that finally process a message received from O_2 sensors and modulate vascular function, and they are (a) enzymes involved in signaling pathways, (b) ion channels, and (c) systems that regulate intracellular Ca^{2+} concentration and the Ca^{2+} sensitivity to the contractile apparatus. No evidence suggests that the effectors are directly modulated by P_{O_2}, but instead, strong evidence supports the hypothesis that their function is modulated by labile factors (like ROS) generated by P_{O_2} sensors. Therefore, we highlight the role of oxidants and redox in relaying signals from the point of O_2 sensing to modulating vascular function.

Soluble guanylate cyclase

Soluble guanylate cyclase (sGC) is an ~140-kDa hemoprotein and a heterodimer consisting of α and β subunits. This enzyme can be directly activated (88) through (a) a heme group on the β subunit that binds nitric oxide in its ferrous (Fe^{2+}) oxidation state (33); (b) oxidation of thiol on cysteine, C243 and C122, respectively in α and β subunits (134); or (c) catalase while it is metabolizing H_2O_2 (23). In contrast, superoxide and thiol redox-dependent mechanisms inhibit the rates of cGMP production (20, 97). It has been known since 1975 that cellular cGMP levels are modulated by P_{O_2} (28). Hence, it is a cellular signaling system regulated by P_{O_2} and by multiple cellular redox systems and ROS. It is evident from the past studies that O_2 does not directly bind to the heme of sGC present in vascular tissue (121). However, all the mechanisms that directly control rates of production of cGMP by sGC are also modulated by redox systems and P_{O_2}-regulated processes. For example, oxygen is required for the biosynthesis of nitric oxide and the ROS that regulate sGC, and it may control other cytosolic NADPH and NADH redox-associated processes that influence sGC activity. Additionally, the heme of sGC appears to be maintained in its ferrous oxidation state by a cytosolic NADPH-dependent methemoprotein reductase activity. This enzyme that is yet to be identified in vascular smooth muscle is vital to prevent oxygen-dependent oxidation of the heme of sGC to its ferric form, known to be resistant to stimulation by nitric oxide in bovine coronary arteries (54). In addition to cytosolic NADPH and NADH being substrates for Nox oxidases generating ROS that potentially activate sGC, NADPH also plays a key role in controlling the redox status of GSH for the metabolism of peroxide that regulates sGC and reverses a thiol oxidation mechanism potentially promoted by GSSG, which seems to inhibit directly the activity of sGC (97). cGMP mediates vascular relaxation through multiple systems that are associated with causing hyperpolarization by opening K^+ channels and reducing intracellular Ca^{2+} concentration and that have also been linked to regulatory systems thought to mediate P_{O_2}-elicited responses. Burke and Wolin (23, 24) provided evidence that hypoxia decreased cGMP in endothelium-removed bovine pulmonary arteries through processes that seem to originate from decreases in peroxide metabolism by catalase. However, the role of this catalase-mediated system in controlling contractile responses was only supported with pharmacologic probes that could potentially have other actions; therefore, further study is needed to establish whether the regulation of sGC is mediating pulmonary artery responses to changes in P_{O_2}. The cGMP system coordinates smooth muscle relaxation through its ability to regulate many processes that control force generation, as a result of activating cGMP-dependent protein kinases (PKG). Some systems that seem to be regulated by cGMP include mechanisms that control the release and reuptake of Ca^{2+} and the sensitivity of the contractile apparatus to Ca^{2+} (66, 83), because PKG directly phosphorylates subunits of the SERCA pump, the inositol 1,4,5-trisphosphate (IP3) receptor, and Ca^{2+}-regulated K^+ channels. Phosphorylation by PKG can potentially increase SR Ca^{2+} reuptake by SERCA, inhibit SR Ca^{2+} release by the IP3 receptor, cause hyperpolarization by opening K^+ channels, and desensitize the contractile apparatus by stimulating myosin phosphatase. In addition, cGMP may function to inhibit the receptor-mediated generation of IP3 by contractile agents. A recent report also suggested that direct activation of PKG due to oxidation of cysteine (Cys42Ser) by H_2O_2 relaxes coronary artery and decreases coronary artery perfusion pressure in rat hearts (22). Thus, when ROS and/or redox systems that regulate the activity of sGC are modulated by changes in P_{O_2}, changes in cGMP levels and PKG activity are likely to result in activation of mechanisms that regulate vascular smooth muscle force.

Ion channels

In excitable cells like VSMCs, generally membrane potential regulates contractility by either opening or closing of ion channels. Therefore, regulation of ion channels by P_{O_2} changes is a primary participant in the modulation of vascular functions. Several ion channels have been shown to be affected by changes in P_{O_2}. Only those channels for which evidence exists that they are directly affected by oxidants and redox are discussed in this section.

K^+ channels. Voltage-gated potassium (K_v) and Ca^{2+}-dependent K^+ (K_{ca}) channels are generally known to maintain resting membrane potential at around –60 mV in coronary artery smooth muscle cells (CASMCs) and PASMCs (109). Consequently, changes in K_v and K_{Ca} channel activity may be critical for modulating smooth muscle membrane potential, L-type Ca^{2+} activity, and contractility. Inactivation of these channels leads to membrane depolarization, which activates inward Na^+ and Ca^{2+} currents and action potentials in VSMCs (109). Both K_v and K_{Ca} channels are expressed in VSMCs; however, a maturational shift occurs in their expression from K_{Ca} to K_v in young to adult PASMCs (127). Consistent with these findings, we detected both $K_{v1.5}$ and big-conductance (B)K_{Ca} channels in calf coronary artery smooth muscle by using immunohisto-

chemistry, but failed to detect any effect of charybdotoxin and iberiotoxin, specific inhibitors of BK_{Ca}, on K^+ currents or on PPP inhibitor–induced relaxation in adult rat PA (52). K_v and K_{Ca} channels are regulated by voltage as well as $[Ca^{2+}]_i$ and voltage, respectively (109). Free radical generation and changes in the cellular ratio of reducing cofactors (NAD^+/NADH, $NADP^+$/NADPH and GSH/GSSG) in VSMCs and oxidizing agents have been proposed to regulate the activity of K_{Ca}, K_{ATP}, and K_v channels and to influence force generation by modulating membrane potential (81, 116, 117, 128). Therefore, these channels are thought to be involved in P_{O_2} sensing and modulating vascular function. Originally, Archer and Weir (8) proposed that redox-sensitive K^+ channels modulated by mitochondrial oxidants might be involved in hypoxic pulmonary vasoconstriction response. Consequently, Post *et al*. (124) identified that K_v currents were reduced by hypoxia in the adult dog PASMCs but not in renal artery smooth muscle cells (RASMCs). Now accumulating evidence from Steve Archer's and Jason Yuan's laboratories (90, 164) supports the role of K^+ channels in the induction of hypoxic pulmonary vasoconstriction and pulmonary hypertension. Recent studies have shown that the β-subunit of the $K_{v1.4}$ and $K_{v1.5}$ channels have NADPH-dependent aldo-keto reductase activity (85, 123), and the β-subunit of BK_{Ca} channel has NADPH-dependent HO-2 activity (168); so that modulation of aldo-keto reductase and HO-1 activity by NADPH/$NADP^+$ ratio could potentially regulate K_v and BK_{Ca} currents (145). Oxidizing agents and intracellular redox potentials, which are presumed to activate K^+ currents by modifying the channel protein or regulating the redox state of a cysteine residue that appears to regulate opening of the ion channel (128, 162), and to activate both K_{Ca} and K_v channels of isolated VSMCs from the rabbit pulmonary and ear arteries (81, 116, 117). In PASMCs and RASMCs, hypoxia induces opening and closing, respectively, of $K_{v1.5}$ channels, an effect mediated by changes in mitochondrial H_2O_2 production (96). Consistent with that finding, we have also found that application of H_2O_2 (100 μM) to bovine CASMC activates tetra-ammonium acetate–sensitive outward K^+ currents. Conversely, our studies suggest that application of G6PD inhibitors paradoxically decreases H_2O_2 levels in CA, and activates both K_{Ca} and K_v channels. In addition, G6PD inhibition, which decreases NADPH-to-$NADP^+$ ratios, relaxes calf CA in H_2O_2-independent manner (50). Opening of K_v channels partially contributes to the relaxation of the adult rat PA and aorta induced by G6PD inhibitors, and inhibition of the G6PD increases outward K_v currents and suppresses hypoxic pulmonary vasoconstriction (52). Furthermore, inhibition of the PPP causes hyperpolarization of normal ferret and chronic hypoxia-exposed human PASMCs through opening of K_{Ca} channels (38, 122). However, data from our previous studies (50, 52) suggest that opening of K^+ channels plays a only minor role in mediating the relaxation of adult rat PA and calf CA elicited by the changes in NADPH and GSH redox.

Ca^{2+} channels. Voltage-gated Ca^{2+} channels are present in most excitable cells. Five high-voltage activated Ca^{2+} channel types (L, N, P, Q and R) and one low-voltage activated channel type (T) are known. L-type Ca^{2+} channels are the primary voltage-gated Ca^{2+} channels in VSMCs (171) and regulate E-C coupling (48). The L-type Ca^{2+} channel exists as a heteromultimer of α_1, β, α_2/δ, and γ subunits, with the voltage-activated Ca^{2+} channel function carried by the α_1 subunits. Recent evidence suggests that the function of the α_{1c} subunit may be modulated by interactions with other cellular proteins. The activity of L-type Ca^{2+} channels is modulated by a variety of neurotransmitters, hormones, and autacoids *via* regulatory processes involving multiple enzymatic reactions. They are also modulated by drugs (*e.g*., dihydropyridines; DHP) that bind directly to the channel protein (62, 111), and in most cases block the channel (65). Ca^{2+} entry through L-type Ca^{2+} channels mediated by DHP-α_{1c} induces Ca^{2+} release from the SR and smooth muscle contraction (14, 72). Glutathione redox, which is directly modulated by NADPH, has been shown to modulate channel function (27, 39) by oxidizing the sulfhydryl group on redox-sensitive cysteine residue present on cardiac myocyte $Ca_V1.2$, the α subunit of the L-type Ca^{2+} channel, and is modulated by dithiothreitol (DTT) [anticipated to increase channel function (50); 3 m*M*], and thiol oxidant, diamide [speculated to suppress Ca^{2+} channel function (69); 1 m*M*]. Interestingly, this channel is also reported to be modulated by changes in P_{O_2}, albeit a heterogeneity exists in the response of voltage-gated Ca^{2+} channels isolated from rabbit conduit *versus* resistant PAs (42, 43, 152). For example, Ca^{2+} channels isolated from conduit PAs close under hypoxia, whereas channels from resistance PAs open by a decrease in P_{O_2}, and this may be one of the mechanisms of initiating hypoxic constriction of the PAs, because contractions to hypoxia are generally most readily detected in resistance-sized PAs. Furthermore, L-type Ca^{2+} channels are also regulated by changes in intracellular NADPH redox, because inhibition of G6PD and decrease in NADPH inactivates L-type Ca^{2+} currents (55). Thus, L-type Ca^{2+} channels are important modulators of VSMC function, and they could be potential targets for oxidants and redox signaling in VSMCs, which requires further investigation to understand fully how they are regulated by P_{O_2} changes.

Sarco(endo)plasmic recticulum. The sarcoplasmic reticulum (SR) is a specialized cellular organelle that sequesters intracellular Ca^{2+} and controls Ca^{2+} levels in the VSMCs either by releasing Ca^{2+} or by reabsorbing Ca^{2+} (uptake of Ca^{2+}) from the cytoplasm. Both these processes are regulated by P_{O_2} and oxidants or redox signaling.

Ca^{2+} is released from ryanodine-sensitive stores *via* ryanodine receptors (RyR2) in VSMCs. Reagents that specifically oxidize free SH groups and promote the formation of disulfide bonds within the RyR2 complex, including 2.2'-dithiodipyridine (DTDP) and 5.5'-dithio-bis(2-nitrobenzoic acid) (DTNB), which activate the channel (176). DTDP activates RyR2 isolated from sheep heart, and this effect is reversed by DTT, which reduces the disulfides to thiols (35). GSH inhibits RyR2 by increasing the binding of calmodulin (CaM) (11). Conversely, O_2^- activates RyR2 by displacing CaM from RyR2 complexes (77). In that regard, recent studies have shown that an oxidoreductase domain is present within the N-terminal of RyR1 in skeletal muscle (10); that NAD(P)H oxidase functions in the SR of coronary arterial smooth muscle; and that O_2^- derived from this NAD(P)H oxidase locally activates RyR-mediated Ca^{2+} release (173). In addition, it has been demonstrated that NADH inhibits RyR2-mediated Ca^{2+} release at physiologic concentrations (IC_{50} = 120 μM) by reducing the open proba-

bility of the channel (26). For example, with increasing workload in intact cardiac myocytes, cytosolic Ca^{2+} diffuses into mitochondria and increases the mitochondrial NADH concentration (17, 93). Mitochondrial shuttle systems for NADH could then potentially increase cytosolic NADH, and this could inhibit RyR2 by a reductive e^- transfer mechanism, which serves as a negative-feedback response to increasing $[Ca^{2+}]_i$ (26). A similar mechanism involving mitochondrial-elicited increases in cytosolic NADH may also regulate RyR2 in vascular smooth muscle in a manner that could be functionally important in transitions between normoxic and hypoxic conditions.

Intracellular Ca^{2+} concentration is also regulated by Ca^{2+} uptake into the internal stores in the SR. This is mainly achieved by SR calcium ATPase pumps, also known as SERCA. Recent studies have shown that Ca^{2+} uptake by SERCA is regulated by S-glutathiolation (4), and the function of SERCA is impaired through the irreversible oxidation of functionally important cysteine residues by oxidants. A mass-spectrometry analysis that identified 18 of a total of 24 cysteine residues in SERCA protein documented that cysteine residue at positions 364, 417, 420, 498, 525, 674, 675, and 938 were modified by oxidative stress (136). Therefore, it is not unreasonable to speculate that modification of cysteine residues by oxidant or redox changes modulated by hypoxia maybe involved in controlling vascular function. In this regard, we have demonstrated that a SERCA inhibitor, cyclopiazonic acid, impairs hypoxic relaxation of human internal mammary and radial artery and bovine CA (56, 58). Additionally, it is known that NADPH redox regulates SERCA function, because thapsigargin and cyclopiazonic acid attenuate relaxation of rat aorta and PA (Gupte and Oka, 2002, unpublished observation) and bovine CA (56) evoked by G6PD inhibitors. Thus, hypoxia appears to regulate the function of SERCA in VSMCs through a mechanism that remains to be defined.

Rho kinase

Another level of Ca^{2+} signaling through which Po_2 changes could act to regulate VSMC function are the processes that control the Ca^{2+} sensitivity to the myofilaments. For example, some of the processes regulated by changes in Po_2 are thought to include kinases such as PKC and Rho kinase that ultimately modulate the activity of enzymes controlling phosphorylation of myosin light chain.

McMurtry and colleagues (106, 108, 113) demonstrated that activation of the Rho kinase signaling cascade, which inactivates myosin light-chain phosphatase, enhances myosin light-chain phosphorylation, potentiates constriction of PA in lung, and evokes hypoxic pulmonary vasoconstriction. Although it is not clear how acute hypoxia activates Rho kinase in the PA, these authors showed that overexpression of Rho kinase by monocrotaline and chronic hypoxia in rats also contributes to the development of pulmonary hypertension (106, 108). As opposed to that in PAs, inactivation of Rho kinase and accelerated dephosphorylation of myosin light chain by acute hypoxia decreasing the Ca^{2+} sensitivity to the contractile apparatus has been proposed as a novel mechanism of relaxation of porcine coronary artery relaxation to hypoxia (110, 156). Because activation of Rho and a subsequent increase in Rho kinase activity mediates ROS-induced Ca^{2+} sensitization and contraction of rat aorta (73), changes in oxidant levels or redox signaling under hypoxia could potentially regulate Rho-Rho kinase systems that are involved in controlling hypoxic constriction and dilatation of pulmonary and systemic arteries, respectively.

It is important to consider the role of endothelium-derived factors like eicosanoids (58) and endothelin (36, 139), as well as cytokines like transforming growth factor-β and bone morphogenetic proteins (BMP) (105) released by chronic hypoxic stimulation, because they could be confounding factors in regulating oxidant production and vascular function.

MODULATION OF HYPOXIC PULMONARY VASOCONSTRICTION AND PULMONARY HYPERTENSION BY OXIDANTS AND REDOX SIGNALING

Oxygen responses are well studied and characterized in arteries from pulmonary as opposed to systemic circulation, as PAs constrict in response to hypoxia to help maintain a balance between perfusion and ventilation in the lungs. This phenomenon, hypoxic pulmonary vasoconstriction (HPV), was observed first in 1876 and later in 1946 by von Euler and Liljestrand. Since then, cellular and molecular mechanisms involved in HPV have been extensively investigated (Fig. 1), but the precise mechanisms responsible for the response are not yet well understood. It is now accepted that a self-regulatory mechanism inherent to the lung controls HPV, because HPV occurs in denervated and explanted lungs and in lung perfused *ex vivo*, therefore excluding neurohumoral effects (120, 130). Furthermore, although the kinetics of sustained HPV remains unresolved, ample evidence suggests that HPV occurs rapidly by a decrease in inspired Po_2 and is switched off quickly by an increase in alveolar Po_2. However, persistent HPV under generalized hypoxia caused by low Po_2 at high altitude or COPD is of significant clinical relevance, because constant hypoxia results in the development of secondary pulmonary hypertension (PH), which is one of the causes of increasing morbidity and mortality in the United States (153). Although several studies implicated chronic HPV, PA remodeling, and inflammation in the development and progression of PH, yet no effective therapies are available to treat PH. Similarly, the cause of idiopathic or primary PH is also not clearly understood, although familial PH is attributed to mutation of the gene encoding BMP-2 receptors and to a BMP signaling malfunction (105, 143). Therefore, identification of the precise pathway involved in evoking primary and secondary PH is imperative to treat this multifactorial disease.

In 1986, Archer and Weir proposed the redox theory based on the studies that suggested that pancreatic K_{ATP} channels, which control insulin release, were redox sensitive and were regulated by mitochondrial-derived H_2O_2 (8). They proposed in their seminal review article in 1986 that mitochondria in PASMCs were the primary Po_2 sensors. Subsequently, these authors suggested that inhibition of free radical production and changes in the ratios of cytosolic reducing cofactors (GSH/GSSG, NADH/NAD^+, NADPH/$NADP^+$) during hypoxia inactivate voltage-gated K^+ ($K_{v1.5}$) channels in PASMCs, resulting in membrane depolarization, Ca^{2+} influx, and vasoconstriction (8, 163). Other studies reported that metabolism of

H_2O_2 by catalase activates sGC, resulting in accumulation of cGMP in bovine PA, and that under hypoxia, H_2O_2 is diminished, leading to a decrease in cGMP and PA constriction (23). Consistently, Archer and co-workers (96) convincingly showed that mitochondrial-derived H_2O_2 regulated $K_{v1.5}$ and membrane potential in PASMCs and RASMCs, although H_2O_2 decreases in the PA but increases in RASMCs with hypoxia (96). They also demonstrated that downregulation of $K_{v1.5}$ expression by sustained hypoxia induces PH and that PASMCs from these animals are unable to sense acute changes in P_{O_2}. However, overexpression of $K_{v1.5}$ channel protein by transfection of adenovirus packaged with adenoviral vector with $K_{v1.5}$ gene insert restores the PA constriction response to hypoxia (125). In the fawn-hooded rat, an anomaly in the mitochondrial–HIF-$K_{v1.5}$ pathway function has also been observed to evoke PH (16). Although it now appears that hypoxic inhibition of the $K_{v1.5}$ channel may contribute to the initiation of HPV, the precise sequence of events that leads to the blockade of K^+ currents is still unclear. It also remains unclear and controversial whether ROS production is decreased or increased during hypoxia (8, 57, 96, 157, 159, 163). Contrary to evidence supporting downregulation of mitochondrial H_2O_2 production evoking HPV and PH, studies have shown that H_2O_2 derived from complex III of the mitochondrial respiratory chain is increased by prolonged/persistent hypoxia in cultured PASMCs (158, 159), PA (79, 84), and lungs (166). Schumacker and co-workers (158) provided convincing evidence for an increase in ROS determined by changes in intracellular redox by using state-of-the-art FRET techniques by hypoxia in cultured PASMCs. It has been suggested that this increase in peroxide contributes to the development of PH by elevating intracellular Ca^{2+} concentrations under hypoxia through increasing the release of Ca^{2+} from internal stores and Ca^{2+} influx through store-operated Ca^{2+} channels (157–161). Additionally, Ca^{2+} influx through TRP channels activated in a redox-sensitive manner also seems to increase intracellular Ca^{2+} concentrations and induces constriction of the PAs (155). In addition to Ca^{2+} influx, compelling evidence indicates that activation of Rho kinase, which is redox sensitive (73), increases the Ca^{2+} sensitivity to the myofilaments of PASMCs and elicits PH (95). Upregulation of Rho kinase expression in monocrotaline- and chronic hypoxia-induced PH appears to contribute to vascular remodeling (106, 108). This system also plays a role in the development of PH in the fawn-hooded rat model (107), and activation of Rho kinase by mitochondrial-derived ROS facilitates constriction of the ductus arteriosus and its functional closure (74). Because inhibition of the Rho kinase signaling cascade prevents as well as reverses PH induced by (a) chronic hypoxia (108), (b) sugen-5416+hypoxia (113), and (c) monocrotaline and pneumonectomized+monocrotaline treatment in rats (95, 106), by decreasing Ca^{2+} sensitivity and preventing vascular remodeling (see Fig. 1), it is considered to be a potential therapeutic target.

Recent studies from our laboratory provided evidence supporting a role for G6PD-derived NADPH in initiating and sustaining HPV (52, 53). Because G6PD activity determined by changes in the NADPH-to-$NADP^+$ ratio in PA and lung is increased by induction of hypoxia (53), and inhibition of G6PD activates K_v function and decreases HPV (52). In addition, the availability of G6PD-derived NADPH controls NADPH oxidase and ROS generation (51), which is involved in mediating hypoxic contraction in bovine PAs (24). By this mechanism, hypoxia is thought to remove a peroxide-mediated relaxation, thereby promoting an HPV response. Consistently, recent studies demonstrated that prolonged treatment with dihydroepiandrosterone (DHEA), a PPP inhibitor, reduces PH (15, 114). Although the mechanism involved in reversing PH with dihydroepiandrosterone is still not clearly understood, activation of K^+ channels by G6PD inhibition (15) and restoration of endothelium-dependent relaxation of PA by an increase in sGC expression (114) or by removal of O_2^-, are potential explanations. Furthermore, increases in NADPH levels in lungs are associated with pulmonary hypertension, and DHEA treatment reduces NADPH levels, PH, and right ventricular hypertrophy (Gupte and Oka, 2005; unpublished data). It is, therefore, evident that oxidants and redox signaling play an important role in PH, and the development of drugs specifically inhibiting oxidant and redox changes that activate signaling, contributing to progression of hypertension (Figs. 1 and 2), might be useful in preventing or treating PH.

OXIDATIVE STRESS, REDOX SIGNALING, AND SYSTEMIC HYPERTENSION

Conceptually, two scenarios exist through which reduction of the P_{O_2} could affect systemic vascular function in primary and secondary hypertension: (a) chronic hypoxia could trigger vasoconstriction and increase blood pressure, or (b) relaxation of systemic arteries, such as coronary or skeletal muscle, to hypoxia could be impaired in hypertension, impeding the blood flow to the stressed organ, and both these conditions would have significant clinical relevance. In this regard, a hypertension task force established by NHLBI in the mid-1970s suggested that metabolic changes due to modulation in P_{O_2} could play a role in controlling autoregulation of systemic arteries and that mechanisms involved in the autoregulation could be altered in hypertension (1). In their report, they noted that if abnormal circulation affects metabolism, and *vice versa*, the potential for positive feedback exists in hypertension, and they recommended initiating more studies to understand behavior of cytochrome aa_3 in normal tissue and regulation of metabolic enzymes by alterations in the P_{O_2} to understand circulation–metabolic coupling *in vivo*. However, not much work on the role of P_{O_2} in controlling vascular function in systemic hypertension has evolved, which is in contrast to extensive studies on the influence of P_{O_2} on pulmonary hypertension.

Typically, coronary and skeletal muscle arteries dilate to maintain the demand–supply ratio to the working heart and skeletal muscle. Lower vascular tone in response to acute hypoxia has been attributed to loss of ATP production, elevation in lactic acid, reduction of pH, increase in adenosine levels, and other metabolic changes (45, 141). As mentioned in earlier sections of this review, hypoxic relaxation is not simply a function of energy stores (34), because the vascular reactivity of arteries is depressed for P_{O_2} in the 20- to 100-torr range, which is higher than the range generally associated with inhibition of mitochondrial oxidative phosphorylation, and the arterial relaxation occurs despite inhibiting respiration with cyanide (30). In porcine CA, despite a marked increase in lactate content, pH_i

is little affected by hypoxia, and neither ATP and P_i levels nor ATP use is altered by acute hypoxia (137). Similarly, no clear evidence exists that changes in NADPH oxidase–derived ROS evoke hypoxic relaxation. For example, studies suggest that NADPH oxidase-derived ROS are decreased by hypoxia, and ROS do not appear to participate in the relaxation of bovine CA induced by an acute decrease in Po_2 (100). Interestingly, we recently demonstrated that G6PD-derived NADPH redox coordinates multiple pathways of reducing the intracellular Ca^{2+} concentration and appears to play a key role in Po_2 sensing and in relaxing bovine coronary arteries to acute hypoxia. It appears that hypoxia has a metabolic effect that inhibits G6PD activity, producing a decrease in the NADPH-to-$NADP^+$ ratio, which evokes relaxation of coronary arteries by decreasing Ca^{2+} influx and release, and accelerating Ca^{2+} uptake by SERCA (56). Therefore, processes summarized in Fig. 2 presumably play a role in relaxing systemic arteries in response to acute hypoxia.

As opposed to acute hypoxia, chronic intermittent hypoxia caused by obstructive sleep apnea (OSA) evokes vasoconstriction and increases systemic blood pressure (12, 21). Studies have found that OSA initially transiently increases night-time blood pressure and eventually evokes a sustained elevation in daytime blood pressure in the conscious OSA dog model (18), and a more recent study in mice showed that the mean arterial blood pressure significantly increases in animals exposed to chronic intermittent hypoxia for 4 months (82). Additionally, studies have found diurnal elevation of blood pressure in rats exposed to repetitive, chronic episodic hypoxia (40, 41). Recent epidemiologic studies, including the Wisconsin Sleep Cohort study and Sleep Heart Health study provide compelling evidence for an association between OSA and hypertension (12, 21). More than 50% of patients with OSA have systemic hypertension, whereas only 25–30% of patients with hypertension have OSA (12). Although mechanism(s) of Po_2 sensing in VSMCs and through which OSA may cause hypertension is/are unclear; it is proposed that activation of hypoxemia, repeated arousal, sustained increases in the catecholamine surge and sympathetic nervous system, and an increase in the renin–angiotensin–aldosterone system activity. This is associated with blunted baroreflex sensitivity with an increase in its set point, elevated endothelin secretion, altered eicosanoid synthesis/activity, increased oxidative stress, and an impairment of endothelium-dependent vasodilatation, which could be potential mechanisms involved in OSA-related hypertension (21). Carotid body chemoreceptors undergo adaptation in response to long-term hypoxia, and this increases peripheral sympathetic neurotransmitter release and elevates blood pressure long after the exposure to hypoxia. Interestingly, patients with hypertension and OSA show greater systemic blood pressure responses to acute hypoxia than do hypertensive patients without apnea (60). However, it is unclear how chemoreceptor-associated signaling is involved in elevating peripheral vascular resistance by increasing sympathetic tone. Recent evidence for elevated ROS production and lipid peroxidation in brain cortical neurons (172), and activation of NADPH oxidase and increased NADPH oxidase subunits $p67^{phox}$ and $p47^{phox}$ overexpression in the lateral basal forebrain, locus ceruleus, dorsal raphe nucleus, hippocampal CA1 pyramidal cells, and cortex of wake-active or hypoxia-sensitive regions of the brain mediate hypersomnolence and brain oxidative injury in a murine model of sleep apnea (175). Therefore, it not unreasonable to speculate that NADPH oxidases act as Po_2 sensors in brain, and increased oxidative stress in the nervous system could be one cause of or mechanism for increasing sympathetic tone and peripheral resistance. Evidence exists for an increase in vascular NADPH oxidase–derived ROS and decreases in NO synthesis or bioavailability in sleep apnea (142), and these changes can potentially mediate constriction of systemic arteries, leading to the development of hypertension in OSA. Overall, the mechanisms through which the intermittent hypoxia associated with OSA promotes hypertension must be further investigated.

Strong evidence exists for the role of ROS in the development of hypertension (146). The activation of NADPH oxidase–derived ROS in Ang II-induced hypertension has been well characterized and documented by several investigators (89, 148). Stimulation of Ang II receptors and G protein–associated PKC signaling pathways activates NADPH oxidase and increases ROS generation in cultured VSMCs and in rat blood vessels, eventually leading to vasoconstriction and hypertension (89). Furthermore, activation of the growth receptor–associated tyrosine kinase/Src kinase-mediated signaling pathways by angiotensin also increases NADPH oxidase-derived ROS generation (135, 147), and this is implicated in increasing blood pressure/systemic hypertension in humans by activating the ERK/MAP kinase pathway. Evidence suggests that increased arterial pressure or stretch itself also activates the production of Nox-derived ROS, which appear to be involved in evoking constriction of multiple vascular segments including coronary arteries (68, 112, 151). Interestingly, knockdown of the Nox-2 isoform of NADPH oxidase by transfection with Nox-2 anti-sense or inhibition of NADPH oxidase by infusion of $gp91^{ds\text{-}tat}$, a competitive and specific inhibitor of Nox-2, ameliorates Ang II–induced hypertension in mice (129), suggesting

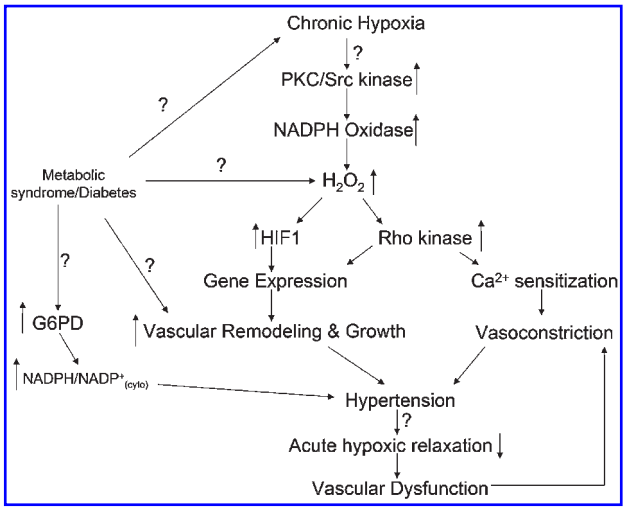

FIG. 3. A schematic that proposes that activation of oxidant production by chronic hypoxia or metabolic syndromes such as diabetes could cause vasoconstriction and hypertension through processes or pathways shown in the model that are known to be redox sensitive. This is hypothesized to reduce responses to acute hypoxia, leading to the progression of hypertension.

that ROS generated by Nox-2 play a predominant role in development of hypertension. Furthermore, recently we found that an increase in Nox-2–derived H_2O_2 elicited by U46619, a thromboxane A_2 analogue, mediates contraction of bovine left anterior descending coronary artery by stimulating CaM-kinase II and Rho kinase (Gupte and Wolin, 2007; unpublished observation); therefore, Nox-2–derived H_2O_2 could potentially induce hypertension by activating both CaM-kinase II and Rho kinase pathways, already known to increase blood pressure (92, 94). Because decreases in the Ca^{2+} sensitivity of force generation by inhibition of Rho kinase is one the mechanisms proposed to mediate hypoxic relaxation (110, 156), activation of Rho kinase by increased ROS generation in hypertension could potentially blunt hypoxic relaxation. Thus, mild or moderate hypertension could eventually cause chronic tissue hypoxia, which is well known to increase neurohumoral mediators (such as endothelin and thromboxane, and activate the renin–angiotensin–aldosterone system). Likewise, an increase in cytokines, such as TNF-α and BMP, activates ROS generation and increases blood pressure (98, 174). Additionally, it is known that BMP is increased by hypoxia (105). Therefore, activation of neurohumoral mediators and cytokines could be hypothesized as a contributing factor to the progression of hypertension, which must be further investigated.

In contrast to evidence supporting a role for oxidants in vascular Po_2 sensing and in the development of hypertension, no clear-cut proof exists for a role for metabolic pathways in Po_2 sensing or hypertension. Although early studies provided evidence suggesting no contribution of an alteration in metabolism and Po_2 in renal hypertension (37), indirect evidence supports a cause–effect relation between alterations in metabolism/ Po_2 and hypertension. It is well known that hypertension is often a confounding factor in diabetic patients (126). A report indicates that small coronary arteries isolated from the atrial appendage of diabetic humans show blunted hypoxic relaxation (99), and internal mammary arteries harvested from diabetic patients undergoing a coronary artery bypass grafting surgery appear to relax poorly under hypoxia (Venkataramana and Gupte, 2007; unpublished observation). Thus, hypertension in combination with metabolic diseases could reduce the VSMC response to changes in Po_2 and impede blood flow to the stressed organs. A change in glucose metabolism is one of the main cause of elevated ROS generation and vascular dysfunction in diabetes (86), and we recently observed that activation of G6PD and increases in NADPH accentuate NADPH oxidase–derived ROS generation, and this impairs endothelium-dependent relaxation and reduces hypoxic relaxation of diabetic Zucker fa/fa rat aorta (Gupte, 2007; unpublished observation). Therefore, existing evidence strongly suggests that hypoxic responses of VSMCs are reduced by hyperglycemia, and processes such as those hypothesized in Fig. 3 to be activated by hypertension should be investigated to define the roles in altering vascular Po_2-sensing mechanisms and their role in promoting tissue hypoxia.

CONCLUDING REMARKS

In summary, several studies have now shown that oxidants and redox signaling play important roles in regulating vascular function in response to changes in Po_2. These oxidant- and redox-related signaling mechanisms involved in Po_2 sensing appear to induce as well as sustain pulmonary hypertension. Although many aspects of systemic vascular Po_2-sensing mechanisms suggest that changes in these mechanisms should be an integral part of the progression of hypertension, little is currently known about their role in this disease process. Further studies are required to understand how the modulation of oxidant and redox changes in VSMCs by hypertension and Po_2-sensing processes contribute to the progression and expression of disease processes to develop new therapeutic approaches to treat or alleviate pulmonary and systemic hypertension.

ABBREVIATIONS

Ang II, angiotensin II; BMP, bone morphogenetic protein; CA, coronary artery; CaM, calmodulin; CaM-kinase II, calmodulin II kinase; CASMCs, coronary artery smooth muscle cells; cGMP, 3'-5' cyclic guanylate monophosphate; DHEA, dihydroepiandrosterone; DHP, dihydropyridines; DTDP, 2.2'-dithiodipyridine; DTNB, 5.5'-dithio-bis(2-nitrobenzoic acid); DTT, dithiothreitol; ERK, extracellular signal-regulated kinase; ETC, electron-transport chain; G6PD, glucose-6-phosphate dehydrogenase; GSH, glutathione; HIF-1, hypoxia-inducible factor; H_2O_2, hydrogen peroxide; HPV, hypoxic pulmonary vasoconstriction; IP3, inositol 1,4,5-trisphosphate; MAP kinase, mitogen-activated protein kinase; Nox-2, NADPH oxidase; O_2^-, superoxide anion; OSA, obstructive sleep apnea; PA, pulmonary artery; PAS, Per-ARNT-Sim; PASMC, pulmonary artery smooth muscle cell; PH, pulmonary hypertension; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PKG, protein kinase; Po_2, partial oxygen tension; PPP, pentose phosphate pathway; PtdInsP, 3-phosphorylated phosphatidylinositol; RASMC, renal artery smooth muscle cell; ROS, reactive oxygen species; RyR2, ryanodine receptor; SERCA, sarco(endo)plasmic recticulum ATPase; sGC, soluble guanylate cyclase; SR, sarco(endo)plasmic recticulum; TF, transcription factor; VSMC, vascular smooth muscle cell.

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