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Forum Review

Cytosolic NAD(P)H Regulation of Redox Signaling and Vascular Oxygen Sensing

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ABSTRACT

This article considers how regulation of signaling controlled by cytosolic NADPH and NADH redox systems contained within the vascular smooth muscle cell may contribute to coordinating alterations in force generation elicited by acute changes in oxygen tension. Additional important issues considered include defining when oxidases generating reactive oxygen species (ROS), such as Nox oxidases, or ROS metabolizing activities which utilize cytosolic NADH and/or NADPH are key participants in eliciting responses that are observed, and assessing how mitochondria can potentially contribute to the regulation that is seen. Many important signaling mechanisms potentially involved in vascular oxygen sensing such as potassium channels, systems regulating intracellular calcium, and the sensitivity of the contractile apparatus to calcium, and the control of cGMP-mediated relaxation by soluble guanylate cyclase appear to be regulated by cytosolic NAD(P)H redox and or ROS. Differences in the processes controlling the maintenance of cytosolic NADPH redox by the pentose phosphate pathway of glucose metabolism are hypothesized to be a key factor in controlling the expression of a relaxation to hypoxia seen in systemic arteries compared to the hypoxic contractile response observed in pulmonary arterial smooth muscle. *Antioxid. Redox Signal.* 9, 671–678.

OXYGEN SENSORS IN VASCULAR REGULATION

There appear to be a number of diverse types of mechanisms which could function as vascular oxygen sensors affecting acute changes in blood flow, and time-dependent alterations in vascular growth and remodeling. For example, the availability of oxygen can influence the generation of tissue-derived metabolites (*e.g.*, adenosine and lactate) and the release of endothelium-derived mediators [*e.g.*, nitric oxide (NO) and eicosanoids] synthesized by oxygenase enzymes, and it can directly affect vascular smooth muscle force (13, 36, 53–55, 60, 61) and growth (7). Control by pO_2 of the generation of reactive oxygen species (ROS), such as superoxide anion and hydrogen peroxide, from sources of these species within the vessel wall and the environment present in the microcirculation is often a major contributing factor to many aspects of regulation that potentially participate in oxygen sensing. Archer and Weir initially highlighted the potential importance of ROS controlling potassium channels opening through modulating vascular smooth muscle cytosolic NAD(P)H and glutathione (GSH) redox as a pO_2 sensing mechanism in hypoxic pulmonary vasoconstriction (2, 3, 28, 29, 35). As discussed in recent review articles (29, 36, 55, 56, 60) since this redox hypothesis was proposed in 1986, data supporting opposing theories on the effects of hypoxia on ROS in vascular smooth muscle and roles for alternative effects of hypoxia on redox signaling have emerged. This article focuses on considering how regulation of signaling controlled by cytosolic NADPH and NADH redox systems contained within the vascular smooth muscle cell may contribute to coordinating alterations in force generation elicited by acute changes in pO_2. Additional, important issues considered include defining when ROS generating oxidase (*i.e.*, Nox oxidases) or ROS metabolizing activities which utilize cytosolic NADH and/or NADPH cofactors as electron donors

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are potentially key participants in eliciting responses that are observed, and assessing how mitochondria can potentially contribute to the regulation that is seen.

REDOX AND THE CONTROL OF SIGNALING

In biological systems, many cellular signaling systems are controlled by one or more redox-regulated components which have functional groups such as thiols or small molecular weight cofactors, including transition metal-containing sites and heme groups whose redox status controls the activity of the protein (12, 27, 59, 60). Each regulated site is likely to have mechanisms controlling its oxidation and reduction. Many aspects of redox control appear to originate from enzymatic processes that generate mediators that shift the redox status of one or more of the components regulating a signaling system containing redox-sensitive sites. Some examples of agents which modulate protein redox toward oxidation include: ROS, nitric oxide (NO) and reactive NO-derived species (NO_x), oxidized lipids, oxidized glutathione (GSSG), and proteins such as oxidized thioredoxin. Some of the reversible oxidation reactions participating in signaling-related processes include: oxidation of protein cysteine thiols (RSH) to form sulfinic acids (RSOH) or disulfides between adjacent protein cysteine thiols (RSSR) or mixed disulfides (termed *S*-thiolation or *S*-glutathionation (RSSG) with the addition of GSH), thiol nitrosation (RSNO) or the disruption of Zn-thiolate and Fe-S centers. Heme binding NO and heme oxidation are also important processes in the regulation of enzymes such as soluble guanylate cyclase (sGC). Peroxides can be mediators of thiol oxidation reactions through their metabolism by GSH peroxidases and peroxiredoxins, resulting in the generation of GSSG and oxidized thioredoxin, which are cofactors controlling enzymatic oxidation of protein thiol groups. Cellular reducing systems controlled by the availability of NADPH are often involved in reductive reversal of oxidant activated signaling through proteins such as GSH and thioredoxin reductases, and electron transport chains associated with heme and transition metal reduction (*e.g.*, cytochrome P450 reductase). Since NADH and NADPH are substrates for oxidases which generate ROS, the availability of these electron donors also influences the generation of oxidants. Thus, the balance between the concentrations of individual oxidizing and reducing systems in subcellular regions is likely to have major roles in determining effects on one or more signaling systems and the expression of physiological responses that are observed.

ORGANIZATION OF CELLULAR REDOX CONTROL MECHANISMS IN VASCULAR SMOOTH MUSCLE POTENTIALLY REGULATED BY HYPOXIA

This review highlights the possibility that some regulatory systems that can be used for linking pO_2 sensing to the control of signaling seem to be organized around metabolic

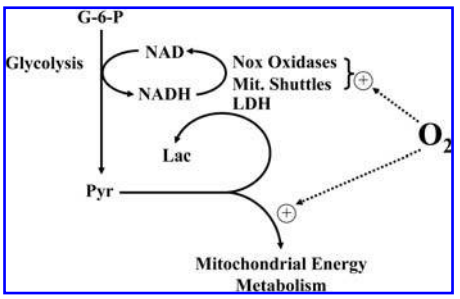

FIG. 1. Sites where O_2 can Influence systems potentially controlling cytosolic NADH redox in vascular smooth muscle. LDH, lactate dehydrogenase.

control of the availability of reductants, including cytosolic NADH and NADPH, and/or oxidants, such as ROS, as key components of these systems. Metabolic processes such as glycolysis and the Krebs' cycle generate NADH in the cytosolic and mitochondrial regions, respectively. In the mitochondria, the redox status or ratio of the reduced NADH and oxidized NAD forms of these cofactors appear to rapidly respond to the balance of multiple processes associated with aerobic and anaerobic energy metabolism, which include ATP consuming cellular work-linked functions and the availability of oxygen. While NADH is needed both for electron transport and the production of mitochondrial-derived ROS, acute changes in mitochondrial NADH/NAD redox have not been viewed as a primary signaling component used in vascular pO_2 sensing. It is thought that the consumption of cytosolic NADH by mitochondrial shuttles and the lactate dehydrogenase reaction results in very low levels of NADH, keeping cytosolic NADH/NAD redox primarily in its oxidized NAD form (Fig. 1). While vascular smooth muscle has high levels of what appears to be NADH-dependent cytochrome b_5 reductase activity (33, 51), a regulatory role for cytosolic NADH redox controlling signaling through this system has not been described. Our studies on the actions of lactate suggest that increasing the availability of cytosolic NADH as a substrate for superoxide generation by Nox oxidases is potentially an important process through which the redox status of this system controls signaling linked to the regulation of vascular force. Lactate has been observed (32, 34, 38, 40, 61) to cause hydrogen peroxide-mediated relaxant responses, which appear to involve increasing cGMP generation by sGC, and enhancement of force generation through activating p44 and p42 mitogen-activated protein kinases (MAPK), which are also known as extracellular regulated kinases (ERK) ERK1 and ERK2, respectively. While hypoxia is expected to increase cytosolic NADH as it impairs mitochondrial metabolism, low pO_2 will also decrease the activities of oxidases. Since the activities of oxidase enzymes are generally thought to be more sensitive to oxygen availability than mitochondrial function, it is difficult to project if ROS production through NADH-dependent oxidase activities will be increased by hypoxia causing cytosolic NADH accumulation or decreased by the limiting availability of oxygen for the production of ROS.

The metabolic control of NADPH redox is less well understood. While several NADH-linked enzymes appear to maintain mitochondrial NADPH in its reduced form, oxidants

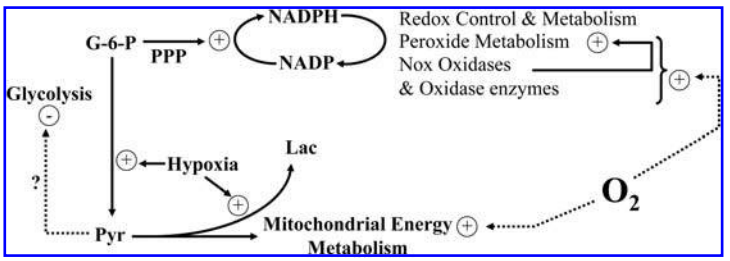

FIG. 2. Sites where O_2 can influence systems potentially controlling cytosolic NADPH redox in vascular smooth muscle.

seem to readily oxidize NADPH, and this has been associated with mitochondrial dysfunction and calcium release from this organelle (6). Most vascular-associated cells appear to use the glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase reactions at the entrance glucose metabolism into the pentose phosphate pathway (PPP) as a primary source of cytosolic NADPH generation. Whereas the contributions of enzymes that oxidize cytosolic NADPH are less well understood, the generation of ROS by Nox or other NADPH-dependent oxidases and metabolism of peroxides by GSH peroxidase and peroxiredoxins are linked to cytosolic NADPH consumption through GSH and thioredoxin reductases. Overall, it appears that a balance of these processes shown in Fig. 2, and perhaps other metabolic enzymes, maintain a partially reduced cytosolic NADPH/NADP redox status in cells and tissues that have been studied. Our studies suggest that the levels of G6PD activity present in vascular smooth muscle may have a major role in controlling the maintenance of cytosolic NADPH levels and the influence of hypoxia on vascular responses that are observed. Bovine pulmonary arteries have higher levels of G6PD and NADPH than coronary arteries from this species, and the elevated levels of NADPH appear to support greater levels of NADPH-dependent Nox oxidase activity than coronary arteries (16). Our previous work in bovine pulmonary arteries suggests that a contributing factor to the contraction caused by hypoxia is a lowering of peroxide and removal of its tonic relaxing effects (9, 10). In addition, our studies on the relaxation to hypoxia seen in bovine coronary arteries suggest that this response is associated with lowering of glucose-6-phosphate (G-6-P) and NADPH levels, suggesting that decreased G6PD activity and NADPH generation by the PPP is an important contributor to the response to hypoxia (18). Interestingly, pyruvate increases G-6-P and NADPH, and inhibits the relaxation observed in bovine coronary arteries to hypoxia, suggesting that metabolic processes controlling function of the PPP have a key role in the pO_2 sensing mechanism. Since hypoxia has been observed to increase the levels of G-6-P and NADPH in isolated rat pulmonary arteries and lungs (19), contractile mechanisms potentially controlled by elevating the ratio of cytosolic NADPH/NADP could be a contributing factor to the contractile response to hypoxia seen.

Localized concentrations or ratios of reduced and oxidized NADH and NADPH in subcellular regions are likely to have important roles in controlling the redox status of other components of redox regulated systems which have a direct influence on signaling. While little is known about the subcellular organization of redox control beyond the cytosolic and mitochondrial regulation of NAD(P)H redox discussed above, there is a wealth of evidence for the clustering of signaling systems in subcellular regions. The subcellular localizations of oxidases generating ROS under conditions where the effects of hypoxia are examined and components of energy metabolism-associated systems maintaining redox control create a potential organization for regulation of redox sensitive components of signaling systems in regions where changes in oxygen tension result in alterations in redox. For example, the loss of maintenance of reduced thioredoxin and GSH as a pool of NADPH is allowed to oxidize is likely to be associated with an accumulation of oxidized thioredoxin or GSSG, which may be cofactors in enzymatic reactions controlling the redox status of protein thiols involved in the regulation of a process such as relaxation or contraction. In addition, the supply of NADPH and NADH as substrates for different forms of Nox oxidases present in subcellular regions seems to be a critical factor in supporting the ROS generating activity of these systems. Thus, redox-linked processes that are potentially important in cellular function could be regulated by the redox status of NADH and NADPH in cellular regions where signaling systems are localized. While many redox-regulated signaling pathways can control vascular smooth muscle force generation, we hypothesize that the organization of cellular redox regulation allows changes in oxygen tension to elicit specific localized alterations in redox signaling associated with the regulation of contractile function.

REGULATORY INTERACTIONS OF CYTOSOLIC NADH AND NADPH REDOX, AND ROS WITH VASCULAR SIGNALING MECHANISMS ASSOCIATED WITH pO_2 SENSING

Metabolic systems controlling cytosolic NADH and NADPH redox are potentially key factors in regulating redox signaling because multiple signaling processes appear to be influenced by the redox status of these cofactors. Several important cellular control or signaling mechanisms that have been associated with modulation by oxygen availability seem to be regulated by ROS and systems linked to cytosolic NADPH and NADH redox. Table 1 lists some of the systems which appear to be important in vascular smooth muscle. Since the organization of these regulatory systems is poorly understood, this review will focus on considering how some of the systems known to be influenced by pO_2 might be regulated by ROS and redox. It is important to realize that redox regulated systems can be controlled by both oxidation and

Table 1. Signaling Systems Modulated by Changes in Oxygen Tension that are Potentially Regulated by Alterations in Cytosolic NAD(P)H Redox and ROS

Target	*Modulators*	*References*
K_v channels	ROS, NADPH/NADP NADH/NAD, GSH/GSSG	17, 28, 41
K_{Ca} channels	ROS, NADPH/NADP NADH/NAD, GSH/GSSG	25, 42
RyR receptors	NADPH/NADP & NADH/NAD	4
IP3 receptors	NADPH/NADP & NADH/NAD	43
SERCA	ROS & GSH/GSSG	1
PKC	ROS	14
sGC	ROS, NADPH/NADP, NADH/NAD, GSH/GSSG	8, 20, 21, 30
Src	ROS	47
Rho/Rho kinase	ROS	23

reduction systems, and that an impairment of a reduction system could enable an oxidative signaling mechanism to be expressed. This section summarizes some of the current understandings of how systems listed in Table 1 seem to be regulated in vascular tissue.

Redox regulation of intracellular calcium ($[Ca^{2+}]_i$) and potassium (K)-channels

Many systems associated with $[Ca^{2+}]_i$ homeostasis and most of the known K-channels either appear to be regulated by redox or have thiols which could be susceptible to a form of redox modulation. In addition, many of the cellular ion transport systems and channels for calcium and potassium in vascular smooth muscle are organized in a manner which enables the systems to interact with each other, making it difficult to define the initial redox event that is potentially regulating multiple systems. Studies on the effects of inhibitors of G6PD and the ability of the PPP to maintain cytosolic NADPH have detected evidence that cytosolic NADPH redox seems to be a key regulator and coordinator of multiple systems that control $[Ca^{2+}]_i$ homeostasis that are shown in Fig. 3 (15). Oxidation of cytosolic NADPH by G6PD inhibitors (*e.g.*, 6-aminonicotinamide, epiandrosterone, and/or dihydroepiandrosterone) promote relaxation through what appears to a combination of stimulating $[Ca^{2+}]_i$ uptake by the sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump, promoting hyperpolarization by opening voltage regulated K-channels (K_v), and preventing vascular contraction by attenuating intracellular Ca^{2+} release and Ca^{2+} influx through ion channels controlled by voltage and receptor regulated mechanisms (15, 17, 18). Regulatory interactions associated with the capacitive filling of the sarcoplasmic reticulum (SR) by Ca^{2+} may allow several of these systems to influence each other through processes not involving alterations in redox. For example, filling of the SR through activation of Ca^{2+} uptake by the SERCA pump may function to both close capacitive-regulated Ca^{2+}-channels and to cause hyperpolarization by opening Ca^{2+}-regulated K-channels (K_{Ca}). Hyperpolarization would also close voltage regulated Ca^{2+}-channels. While SERCA has thiol sites whose oxidation stimulates SR Ca^{2+}-uptake (1), the redox processes regulating signaling mechanisms around the control of $[Ca^{2+}]_i$ are less well understood. Based on studies in other types of muscle (4, 43), systems releasing Ca^{2+} from the SR associated with receptors for inositol triphosphate (IP3) and ryanodine (RyR) are additional systems which could potentially be regulated by changes in cytosolic NAD(P)H redox. Oxidants such as hydrogen peroxide have also been observed to elicit contraction in vascular preparations, and some of the redox regulated contractile mechanisms are considered below. Control of the regulation of cytosolic NADPH redox by pO_2 is a key component of several of the theories on how hypoxia regulates force in both pulmonary and systemic arteries, and there is general agreement in the theories considering this issue that cytosolic NADPH oxidation is associated with decreased force generation or relaxation (2, 3, 17–19, 28, 29, 36, 60).

Soluble guanylate cyclase

The product of sGC, cyclic GMP, is a second messenger which coordinates multiple processes mediating vascular relaxation (22, 26). Several redox and ROS associated processes seem to control the generation of cGMP by sGC. NO stimulates sGC activity by binding the ferrous heme of this enzyme. The ability of NO to stimulate sGC and vascular relaxation are impaired by oxidation of the heme of sGC and by what appear to be a thiol oxidation processes regulated by the formation of GSSG (21, 30, 49). Cytosolic NADPH generated by G6PD and the activity of the PPP also appears to maintain the heme of sGC in its ferrous form and to prevent the accumulation of oxidized GSSG, enabling sGC to be

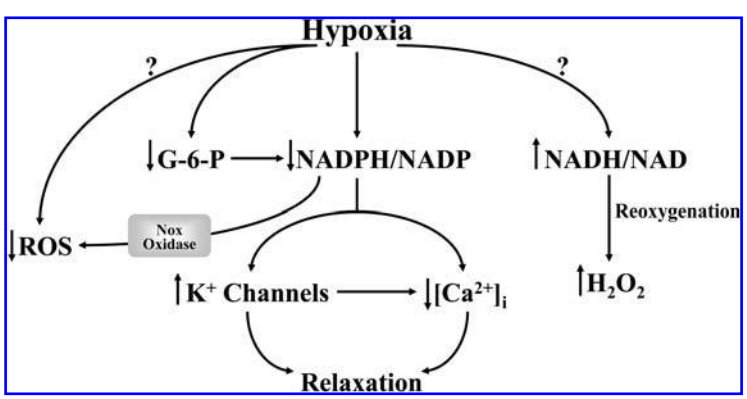

FIG. 3. Hypothesized role of cytosolic NADPH oxidation in coordinating the relaxation of systemic arterial smooth muscle to hypoxia.

stimulated by NO and perhaps other activators (21). Interestingly, hypoxia has been observed to enhance the sensitivity of sGC in pulmonary arteries to activation by NO (31) in a manner that appears to be associated with increases in NADPH elicited by hypoxia (19), and this may contribute the influence of NO in modulating hypoxic vasoconstriction in lungs (44, 45). Superoxide and hydrogen peroxide generated from pO_2 regulated sources including cytosolic NAD(P)H-linked Nox oxidases appear to have a major influence on sGC activity (16, 20, 34, 60, 61). Superoxide is an extremely reactive scavenger of NO, and it may also have additional effects associated with inhibition of sGC stimulation. Hydrogen peroxide can be a stimulator of sGC, and the metabolism of peroxide by catalase appears to enable peroxide to stimulate sGC in vascular and purified enzyme preparations (8, 11, 58). Several studies have identified vascular responses elicited by changes in pO_2 that appear to be mediated by changes in sGC activity through alterations in the level of ROS and/or NO (9, 10, 19, 35, 44, 45).

Redox regulated contractile signaling systems

Studies on the actions of ROS and agents generally thought to modulate thiol redox have identified many signaling systems which can potentially be regulated by pO_2 through redox-associated processes. Increased activities of protein kinase C (PKC), Rho kinase, and MAPK, including ERK1, ERK2, and p38 MAPK, have all been associated with enhancement of force generation. The precise mechanisms and sites through which each of these systems is regulated by oxidants are rather poorly defined. There is evidence in vascular smooth muscle that oxidants activate PKC systems and inhibit tyrosine phosphatases (27, 47). Processes that may involve the oxidation of Zn-S centers in a manner which is modulated by the thioredoxin system have been associated with activation of different forms of PKC (14, 24). In addition, oxidants have been shown to stimulate multiple phospholipase enzymes which could result in the release of lipid regulators of PKC (*e.g.*, diacylglycerol) and other signaling systems (37). The activation of PKC also seems to be an important signaling system for activation of ROS production by p47phox- and rac-regulated Nox oxidases (47). Rho kinases have been observed to be stimulated by mechanisms regulated by oxidants, suggesting that the redox regulation of these other systems could control Rho kinase activity and its force enhancing actions which have been linked to the inhibition of myosin light chain phosphatase (23). A peroxide stimulated src kinase-EGF receptor mechanism has been observed to promote activation of ERK1/2 (39), which may function to enhance force through phosphorylation of the actin-associated regulatory proteins caldesmon and calponin. Oxidants also promote p38 MAPK kinase activation (52), and this system may also influence force generation. The src kinase system is an oxidant regulated signaling system which could influence contractile function through multiple mechanisms including its role in promoting rac-mediated activation of Nox oxidases (47) and/or EGF receptor linked activation of ERK1/2 (39). Thus, oxidants seem to regulate many signaling systems which influence force in a manner that could contribute to the regulation of vascular function by changes in oxygen tension. Since many of the processes discussed in this section seem to be more associated with physiological adaptation and/or pathophysiology, they may have a greater role in vascular remodeling than in the acute responses of vascular tissue to changes in oxygen tension.

DOES THE STATUS OF CYTOSOLIC NADH AND/OR NADPH REDOX FUNCTION AS KEY COORDINATORS OF VASCULAR SMOOTH MUSCLE RESPONSES TO ACUTE CHANGES IN pO_2?

Many of the theories on how pO_2 is sensed in vascular tissue can be viewed as focusing on modulation of ROS generation or redox-linked signaling which can be associated with their influence on the control of cytosolic NADPH, with cytosolic NADH redox also potentially contributing to the regulation that is observed. While much attention has been focused on the role of changes in ROS generation by mitochondria in pO_2 sensing, the function of this organelle has only been associated with a few types of regulatory processes beyond releasing ROS such as hydrogen peroxide, which can be linked to signaling mechanisms controlling vascular smooth muscle force. Since there is little evidence for limitations in energy metabolism or mitochondrial inhibitors mimicking the effects of hypoxia by limiting energy metabolism (18, 48, 53), this primary role for mitochondria in cell function does not appear to be a key process contributing to the regulation of force in response to acute changes in pO_2. Some of the most well documented signaling effects of mitochondria are sensing cytosolic calcium for the matching of cellular work to energy metabolism, preventing localized high concentrations of intracellular calcium and cell death-associated signaling. However, these processes have not been associated with the regulation of vascular function elicited by acute changes in pO_2. Several of the major theories on vascular regulation by hypoxia emphasize the influence of changes in pO_2 on mitochondrial ROS generation (55–57, 60), and the control of cytosolic redox by the metabolism of mitochondrial-derived hydrogen peroxide is a key component of the redox hypotheses proposed for hypoxia-elicited regulation of K-channels and force in both pulmonary and systemic arterial smooth muscle by Archer and colleagues (29, 36).

Our studies suggest that cytosolic NADPH redox is a coordinator of multiple processes lowering intracellular calcium (see Fig. 3) as increased oxidation to NADP occurs, and that changes in pO_2 regulate NADPH redox in vascular tissue. Bovine coronary arteries show an oxidation of this pool, associated with a decreased NADPH to NADP ratio (18), when exposed to hypoxic conditions that promote relaxation. In contrast, rat pulmonary arteries show an increased NADPH-to-NADP ratio on exposure to hypoxic conditions that elicit vasoconstriction (19). Some of the studies on pulmonary artery responses to hypoxia have documented decreases in hydrogen peroxide with hypoxia. As shown in Fig. 4, hypoxia could be raising cytosolic NADPH levels in pulmonary arteries by both inhibiting the consumption of cytosolic NADPH by Nox oxidases used for the generation of peroxide,

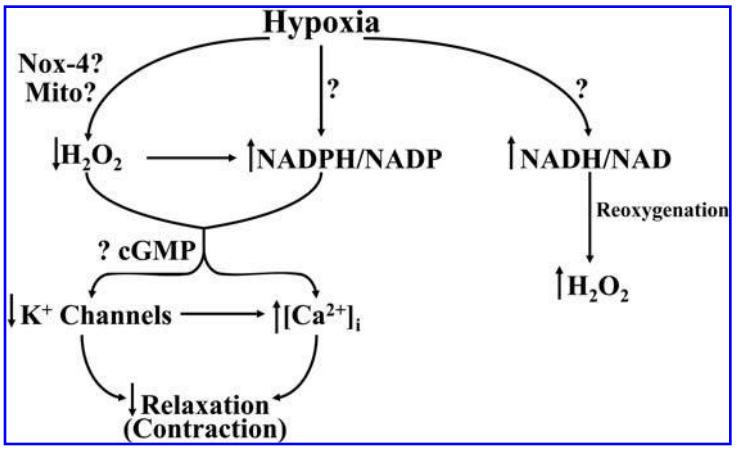

FIG. 4. Hypothesized roles for decreases in ROS and increases in cytosolic NADPH in coordinating the contractile response of pulmonary arterial smooth muscle to hypoxia.

and the consumption of NADPH by peroxide metabolizing enzymes as hypoxia decreases the levels of peroxide derived from either Nox oxidases or other sources such as mitochondria. For example, the angiotensin II stimulation of Nox oxidases is associated with a decrease in cytosolic NADPH levels (5). Our studies suggest that higher levels of the PPP enzyme G6PD in bovine pulmonary arteries compared coronary arteries may function to both maintain elevated levels of NADPH in the pulmonary system and to prevent the oxidation of NADPH caused by hypoxia in coronary arteries (16, 18). In addition, the higher levels of cytosolic NADPH in the pulmonary arteries appear to result in increased ROS production, and the maintenance of a tonic relaxation by hydrogen peroxide which is attenuated by hypoxia. When peroxide is formed, it could be causing relaxation by eliciting thiol redox changes on sites such K^+ channels (29, 36), or stimulating sGC to promote a coordination of relaxing mechanisms through the cGMP system (60). Since hypoxia appears to promote increased release of lactate from smooth muscle under hypoxia conditions (50), cytosolic NADH may be increased under hypoxia. However, the potential regulatory role of an increase in cytosolic NADH controlling smooth muscle force under hypoxia is not understood. Since reoxygenation after exposing vascular smooth muscle preparations to hypoxia elicits transient relaxation or contractile responses that appear to be mediated through a transient elevation of peroxide upon reoxygenation, increases in cytosolic NADH during hypoxia hypothesized in the models shown in Figs. 3 and 4 could be a contributing factor to increases in ROS seem upon reoxygenation. There is evidence that hypoxia increases the basal effects of NO-mediated relaxation in the pulmonary vasculature in a manner which is associated with it functioning to suppress the full expression of hypoxic pulmonary vasoconstriction (44,45). Interestingly, it has been observed that hypoxia improves the efficiency of NO stimulation of sGC in pulmonary arteries associated with increased levels of NADPH (19). Thus, the sGC system in pulmonary arterial smooth muscle may have multiple roles in controlling expression of hypoxic pulmonary vasoconstriction.

Observations that pyruvate attenuates relaxation of bovine coronary arteries to hypoxia (18) and functional increases in microvascular blood flow (62) may provide insight into both the mechanisms involved and the physiological importance of cytosolic redox systems in the metabolic control of blood flow. Pyruvate increased G-6-P and NADPH in coronary arteries in a manner which attenuated decreases in these metabolites and relaxation elicited by hypoxia. These observations suggest (see Figs. 2 and 3) that a key factor in the sensing of hypoxia by systemic arteries may originate from stimulation of increases in glycolysis by hypoxia potentially impairing the ability of G6PD and the PPP to maintain cytosolic NADPH. While it is not known if a decrease in pyruvate by hypoxia contributes to the decreased level of G-6-P that are seen, hypoxia has been observed to lower smooth muscle pyruvate levels (50). Although endothelium- and tissue-derived mediators have been the focus of many previous studies on microvascular regulation by hypoxia, recent studies have been providing evidence that several mechanisms involving alterations in cytosolic redox (62) and ROS (46) may be important factors in the metabolic or functional regulation that is observed. Coronary arterioles appear to be exposed during hypoxia to levels of lactate that cause vasodilation, and an agent (dichloroacetate) that decreases lactate by promoting mitochondrial pyruvate consumption attenuates the vasodilation to hypoxia that is observed (13). It is possible that both increases in cytosolic NADH and ROS, and decreases in cytosolic NADPH, are contributing to the arteriolar dilation that is observed. Hydrogen peroxide appears to be released from cardiac tissue in a manner associated with myocardial oxygen consumption, and infusion of catalase attenuates increases in blood flow associated with myocardial work that are observed (46). Thus, a combination of processes causing alterations in arteriolar smooth muscle cytosolic NAD(P)H redox, including its exposure to hypoxia and tissue-derived peroxide and lactate, may be major factors in the metabolic regulation of blood flow associated with matching tissue demand for oxygen with the control of blood flow associated with its delivery.

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ABBREVIATIONS

ERK, extracellular regulated kinases; G6PD, glucose-6 phosphate dehydrogenase; GSH, glutathione; GSSG, oxidized glutathione; MAPK, mitogen-activated protein kinases; NO, nitric oxide; NO_x, reactive NO-derived species; ROS, reactive oxygen species; RSH, protein cysteine thiols; RSNO, thiol nitrosation; RSOH, sulfinic acids.

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