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Redox Regulation of Guanylate Cyclase and Protein Kinase G in Vascular Responses to Hypoxia

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Abstract

The production of cGMP by the soluble form of guanylate cyclase (sGC) in bovine pulmonary arteries (BPA) is controlled by cytosolic NADPH maintaining reduced thiol and heme sites on sGC needed for activation by NO, and the levels of Nox oxidase-derived superoxide and peroxide that influence pathways regulating sGC activity. Our recent studies in BPA suggest that the activities of peroxide metabolizing pathways in vascular smooth muscle potentially determine the balance between sGC stimulation by peroxide and a cGMP-independent activation of cGMP-dependent protein kinase (PKG) by a disulfide-mediated subunit dimerization. Cytosolic NADPH oxidation also appears to function in BPA through its influence on protein thiol redox control as an additional mechanism promoting vascular relaxation through PKG activation. These processes regulating PKG may participate in decreases in peroxide and increases in NADPH associated with contraction of BPA to hypoxia and in cytosolic NADPH oxidation potentially mediating bovine coronary artery relaxation to hypoxia.

Keywords

guanylate cyclase; hypoxia; NADPH redox; peroxide metabolism; thiol redox

1. Introduction

Regulation of cGMP-dependent protein kinase (PKG) activity is potentially an ideal system for use by oxygen sensing mechanisms in controlling vascular smooth muscle function, because multiple redox controlled processes potentially influence the role of PKG as a coordinator of signaling mechanisms that promote vascular relaxation. For example, redox processes could influence the expression and activities of cGMP production by guanylate cyclase, the removal of cGMP by phosphodiesterases, and protein phosphorylation processes regulated by PKG. While many of the mechanisms that control intracellular calcium and its action on the contractile apparatus have been associated with redox regulation in vascular responses to hypoxia (Moudgil, et al., 2005; Waypa and Schumacker, 2005; Wolin, et al., 2005), minimal information is available to evaluate if PKG is contributing to regulating these processes. Data reported in the literature have resulted in major of controversy regarding the source of redox changes resulting from exposure of pulmonary and systemic arteries to hypoxia. For example, theories exist for hypoxia eliciting either increases or decreases in reactive oxygen species originating from vascular

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smooth muscle sources such as mitochondria and/or Nox oxidases being the origin of redox changes (Moudgil, et al., 2005; Waypa and Schumacker, 2005, Wolin, et al., 2005). Due to the possibility of the experimental conditions causing adaptations in how hypoxia regulates redox processes, we have focused on studying the effects of hypoxia under conditions where pulmonary arteries contract and coronary arteries relax upon exposure to hypoxia. Under these conditions, our studies in bovine pulmonary arteries have provided evidence for the oxygen dependence of hydrogen peroxide production by Nox oxidases functioning as an oxygen sensor regulating cGMP production by soluble guanylate cyclase (sGC) and PKG activity in the acute response of these arteries to hypoxia (Burke and Wolin, 1987; Ahmad, et al., 2010). In contrast, hypoxia appears to cause coronary arterial relaxation through a peroxide-independent mechanism coordinated by the oxidation of cytosolic NADPH (Gupte and Wolin, 2006). Since the activity of soluble guanylate cyclase (sGC) is directly regulated by multiple reactive oxygen species (ROS), thiol and heme redox processes (Wolin, et al., 2005), and PKG has recently been shown to be activated by peroxide (Burgoyne, et al., 2007), this review focuses on examining how the properties of these systems could participate in vascular responses to hypoxia.

2. Early evidence for the redox regulation of guanylate cyclase

Many of the early observations related to the redox regulation of sGC provide evidence for how this system can function in the signal transduction mechanisms associated with oxygen sensing. One of the first observations reported in 1976 suggested that redox processes may influence the cGMP signaling system. This study showed that incubation of the 37,000 x g centrifugation supernatant fraction of homogenized rat lungs resulted in an oxygen-dependent increase in soluble guanylate cyclase (sGC) activity, which was inhibited by thiol reductants (White, et al., 1976). Hydrogen peroxide was found to reproduce the autooxidation-type effects of incubation suggesting that it was an activator of sGC (White, et al., 1976). While copper binding agents inhibited the activation of sGC on incubation, scavenging superoxide with added SOD did not alter activation, and modulation of catalase activity had both stimulatory and inhibitory effects on sGC activity (White, et al., 1976). Based on our initial studies on how ROS interact with sGC activity in the bovine pulmonary artery (Burke and Wolin, 1987) and our more recent work (Ahmad, et al., 2009, 2010), these effects could be interpreted as endogenous catalase participating in the activation of sGC while it was metabolizing peroxide. Peroxide was generated from endogenous superoxide by Cu,Zn-SOD activity present in the lung homogenate. Studies that began with a report in 1975 that sodium azide stimulated sGC activity (Kimura, et al., 1975) evolved into recognizing that many nitrogen oxide-containing substances and nitrovasodilator drugs were being metabolized by a variety of enzymatic and non-enzymatic reactions into nitric oxide (NO) (Anderson, et al., 1994; Kelly and Smith, 1996), which functioned as a direct activator sGC (Craven and DeRubertis, 1978; Katsuki, et al., 1977). There were also early reports that biological substances such as dehydroascorbate, lipid peroxides, SOD and hydroxyl radical were stimulators of sGC activity (Graff, et al., 1978; Haddox, et al., 1978; Mittal and Murad, 1977). In addition, a variety of chemical oxidants and reductants used in early studies to probe cellular redox processes were also reported to influence sGC activity (Arnold, et al., 1977). While the origins of many of these observations have not been sorted out, it is likely that a combination of alterations in ROS, their interaction with NO and changes in redox cofactors directly influencing sGC activity (e.g. sGC thiols and heme) contributed to these early observations. It was also reported in 1975 that hypoxia regulated cGMP levels in human umbilical veins (Clyman, et al., 1975) While the mechanisms responsible for this observation are not known, it could be hypothesized that the generation of NO and/or ROS could be contributing factors. Thus, sGC appears to be regulated by multiple redox mechanisms that could contribute to how oxygen sensing regulates vascular function.

3. Redox aspects of the regulation of sGC by nitric oxide

Many aspects of how NO regulates sGC have redox regulated components which could influence vascular responses to changes in oxygen tension. Initially, purification of sGC led to evidence that the amount of Fe^{2+}-heme remaining bound to sGC was a key factor in enabling NO activation (Craven and DeRubertis, 1978; Gerzer, et al., 1981; Ignarro, et al., 1982). The influence of thiols on sGC regulation by NO is complex due to their involvement in the metabolism of drugs releasing NO, in modulating the ROS and NO oxidation products and in altering the redox of thiols influencing sGC activity. Reduced thiols generally support NO-mediated activation of sGC. While the role of heme in sGC regulation was debated, there was a hypothesis that S-nitrosothiols (RSNO) were the key activators of sGC derived from NO and NO-related agents (Ignarro, et al., 1981), and recent studies have provided support for an alternative mechanism of sGC activation via RSNO (Fernhoff, et al., 2009). An extremely important biological property of NO is that it reacts with superoxide at diffusion controlled rates, which exceed the rate of reaction of superoxide with the various forms of SOD (Pryor and Squadrito, 1995). Thus, at the levels of NO in the range of 1–100 nM which stimulates sGC activity in vascular smooth muscle, increases in biological levels of superoxide typically function to attenuate the actions of NO. As NO levels approach the tissue levels of SOD, NO will become a significant scavenger of superoxide generating peroxynitrite in amounts that are likely to have biological effects such as oxidizing and nitrating thiols, oxidizing the heme of sGC and irreversibly inhibiting mitochondrial respiration (Davidson, et al., 1997; Stasch, et al., 2006; Xie and Wolin, 1996). The oxygen-dependence of endothelium-derived NO biosynthesis and superoxide sources influencing NO signaling can be important factors in vascular responses elicited by changes in oxygen tension (Prieto, et al., 2010). Thus, sGC regulation by NO is influenced by multiple redox controlled processes that could influence how oxygen sensing regulates vascular function.

4. Influence of reactive oxygen species on the regulation of sGC

Early studies detecting evidence for redox regulation of sGC suggested ROS could be major factors in controlling sGC activity. While elevated levels of superoxide function to attenuate the actions of NO, basal levels of superoxide normally being metabolized by various forms of SOD have the potential to regulate sGC activity through the formation of hydrogen peroxide. Hydrogen peroxide appears to stimulate sGC while it is being metabolized by the enzyme catalase associated with levels of peroxide that form the compound I intermediate of catalase (Burke and Wolin, 1987). SOD seems to be an essential component of this process as a result of the need for this enzyme to efficiently convert superoxide into peroxide (Ahmad, et al., 2009, 2010; Burke-Wolin and Wolin, 1989; Burke and Wolin, 1987). Since catalase appears to be inhibited by both NO and superoxide (Mohazzab-H, et al., 1996), these may be additional factors in controlling when sGC regulation by peroxide will be detected. While carbon monoxide (CO) has been reported to be a weak activator of sGC (Brune and Ullrich, 1987; Furchgott and Jothianandan, 1991), a more dominant action of CO could be its ability to stimulate ROS formation in tissues (Lamon, et al., 2009). At higher levels of ROS generation, it is likely that the ability of NO to stimulate sGC will also be impaired by oxidation of sGC heme and/or thiols (Wolin, 2009), and peroxynitrite appears to readily promote sGC heme oxidation (Stasch, et al., 2006). Thus, changes the ROS present and their interaction with NO that occur during physiological and pathophysiological processes may alter oxygen sensing mechanisms regulating vascular function through PKG and other redox regulated systems.

5. Hydrogen peroxide metabolism functions as activator of both sGC and PKG-Iα

Redox changes that result from the metabolism of hydrogen peroxide (H_2O_2) potentially function as very sensitive sensors of changes in peroxide levels through the regulation of sGC and PKG. H_2O_2 is metabolized by several enzymes such as catalase, glutathione peroxidase and peroxiredoxins, with majority of H_2O_2 being consumed by glutathione peroxidase generating oxidized glutathione (GSSG) and water as end products. As shown in Fig. 1, GSSG in turn, acts as a substrate for enzymes that may regulate thiol redox of proteins (Forman, et al., 2004;Wolin, 2000). Catalase and peroxiredoxins are likely to be significant contributors to H_2O_2 metabolism in the subcellular regions where they are enriched compared to the activities of glutathione peroxidases. H_2O_2 metabolism by catalase generates an intermediate of catalase termed compound I that appears to mediate the activation of sGC. The metabolism of peroxide by peroxiredoxins generates oxidized thioredoxin, and thioredoxin reductase/thioredoxin systems are well established as protein thiol redox regulators (See Fig. 1). Simultaneously, H_2O_2 is also capable of promoting oxidation of thiol groups only on the PKG-Iα monomers and not on the PKG-Iβ monomers, forming an inter-subunit disulfide bond, activating the kinase independently of cGMP (Burgoyne, et al., 2007). Although there are hypotheses assuming peroxides interact with signaling systems by directly reacting with thiol groups in their more reactive anionic form, the slow rates that have been reported for these reactions suggest alternative processes may be involved. In BPA precontracted with serotonin (incubated under hypoxia to lower endogenous H_2O_2) and exposed to increasing concentrations of H_2O_2, we observed that 0.1–1 mM H_2O_2 caused increased PKG dimerization and relaxation (Neo, et al., 2010b). These responses were associated with increased phosphorylation of vasodilator-stimulated phosphoprotein (VASP) at the serine-239 site known to be mediated by PKG. Treatment of BPA with 1 mM dithiothreitol (DTT) attenuated PKG dimerization, VASP phosphorylation and relaxation to H_2O_2. In addition, depletion of sGC appeared to attenuate ~60% of the increase in VASP phosphorylation and relaxation of BPA to 0.1 mM H_2O_2, without altering PKG dimerization. Thus, both sGC/cGMP-dependent PKG activation and cGMP-independent thiol oxidation-elicited dimerization activation of PKG appear to be a contributing factor to the relaxation of BPA to H_2O_2. Since PKG dimerization has been detected by our laboratory in bovine coronary and pulmonary arteries, mouse aorta and pulmonary arteries and by others in rat coronary arteries (Burgoyne, et al., 2007) and human coronary arterioles (Zhang, et al., 2009) it may function as fundamental vasodilator mechanism in other segments of vascular smooth muscle. Thus, H_2O_2 metabolism functions as activator of both sGC and PKG-Iα. While the cGMP generated by sGC can potentially activate both forms of PKG, only the PKG-Iα form is expected to be activated by thiol oxidation mediated dimerization independent of cGMP.

Despite the different modes of activation of the PKG, both forms of this kinase potentially function as a cohesive coordinator, promoting events that ultimately give rise to H_2O_2 mediated vasorelaxation. PKG appears to lower intracellular calcium through various processes including stimulation its uptake by the SERCA pump, promoting hyperpolarization by opening K-channels and inhibiting the generation of inositol triphosphate needed for release of intracellular calcium (Lincoln, et al., 2001). In addition, the phosphorylation of Rho kinase by PKG attenuates its inhibition of myosin phosphatase, a cGMP-mediated process which is thought to desensitize the contractile apparatus to the actions of calcium. Thus, the PKG system when activated by cGMP-dependent and/or cGMP-independent mechanisms is designed to coordinate relaxation of various contractile processes through both lowering intracellular calcium and inhibiting its actions on the contractile apparatus.

6. Role of cytosolic NADPH in the control of sGC and PKG activation by thiol oxidation mediated subunit dimerization

The status of cytosolic NADPH redox is potentially an important regulator of sGC and PKG which could influence signaling mechanisms associated with vascular oxygen sensing. We have previously provided evidence that inhibition of glucose-6-phosphate dehydrogenase (G6PD) in the pentose phosphate pathway promotes oxidation of NADPH, and this elicits vascular relaxation through coordinating multiple processes that lower intracellular calcium (Gupte, et al., 2003). In addition, cytosolic NADPH functions as a central switch to regulate redox state of heme and thiols of sGC and thiols of PKG (See Fig. 1).

Elevated cytosolic NADPH appears to maintain the redox status of sGC heme and thiols in a manner which enables NO to stimulate the generation of cGMP. A cytosolic, NADPH-dependent flavoprotein-containing methemoprotein reductase system seems to be required for maintaining the redox status of the heme of sGC in its ferrous (Fe^{2+}) NO-binding form (Gupte, et al., 1999) enabling NO to promote vascular relaxation through increasing cGMP. For example, the sGC heme oxidant ODQ or inhibition of methemoprotein reductase activity results in an attenuation of BPA relaxation to NO. Both the thiol oxidant diamide and G6PD inhibition with 6-aminonicotinamide (6-AN) were observed to attenuate NO-elicited relaxation of BPA and stimulation of sGC activity in homogenates obtained from arteries treated with these agents (Mingone, et al., 2006). Treatment of BPA with the thiol reductant DTT (1 mM) reversed inhibition of NO-mediated relaxation and sGC stimulation by 6-AN, but it did not reverse inhibition by heme oxidation with ODQ, suggesting that a dominant effect of G6PD inhibition on sGC regulation is associated with promoting thiol oxidation. Cytosolic NADPH generation by the pentose phosphate pathway (PPP) presumably restores heme in its reduced state, preserving relaxation to NO in the presence of the heme oxidants. In addition to the heme redox mediated control of sGC activity, there are thiols on sGC that help to maintain its activity. Thiols are thought to be essential for the generation of cGMP by sGC, and incubation of sGC with oxidized thiols has been observed to inhibit sGC by an *S*-thiolation (Brandwein, et al., 1981; Tsai, et al., 1981). In the presence of diamide, we detected an inhibition of NO-mediated relaxation and activation of sGC in arterial homogenates from arteries treated with this thiol oxidant. Thus, a thiol oxidation mechanism functions to inhibit relaxation to NO by attenuating the ability of NO to activate sGC in BPA. Cytosolic NADPH in cooperation with thiol reducing/oxidizing enzymes restore thiols on sGC to their reduced state, preserving relaxation to NO in the presence of thiol oxidants. A GSSG-dependent enzymatic reaction appeared to cause inhibition of sGC stimulation by NO, suggesting glutathione redox may regulate the control of cGMP generation by NO (Mingone, et al., 2006).

In our recent studies, we hypothesized that cytosolic NADPH may also function to regulate PKG through maintaining its thiols in their reduced state (See Fig. 1). Inhibitors of G6PD (1 mM 6-AN and 0.5 mM epiandrosterone (Epi)) were observed to increase PKG dimerization in BPA precontracted with 20mM KCl under hypoxic conditions where relaxation was observed (Neo and Wolin., 2010). These responses were associated with increased phosphorylation of vasodilator-stimulated phosphoprotein (VASP) at the serine-239 site known to be mediated by PKG. Thus, inhibition of G6PD promotes a thiol oxidation-elicited dimerization activation of PKG, suggesting that PKG activation is likely to be a participant in mechanisms through which cytosolic NADPH oxidation promotes vascular relaxation. As hypothesized in Fig. 1, oxidized thioredoxin and GSSG are potential common intermediates in possible pathways controlling the thiol oxidation-mediating dimerization. If GSSG is the common intermediate, proteins such as glutaredoxin or possibly protein disulfide isomerase (PDI) may be participants in catalyzing the thiol oxidation-mediating dimerization (Forman, et al., 2004). Thus, the balance between various systems regulating thiol redox that are

controlled by cytosolic NADPH redox are likely to have a major influence on the properties of regulation through sGC and PKG under diverse physiological and pathophysiological conditions in a manner which could determine the processes involved in vascular oxygen sensing mechanisms.

7. Evidence for potential roles for the redox regulation of soluble guanylate cyclase and protein kinase G in vascular responses to hypoxia

Studies on the role of ROS in vascular responses to hypoxia potentially related to the regulation of sGC have detected evidence for superoxide interacting with NO (Prieto, et al., 2010) and hypoxia controlling the generation of peroxide which is derived from metabolism of superoxide by SOD (Ahmad, et al., 2009; Burke-Wolin and Wolin, 1989; Mohazzab-H and Wolin, 1994). In addition, the control of cytosolic NADPH generation by G6PD and the pentose phosphate pathway of glucose metabolism also seem to be a major factor in controlling responses to hypoxia (See Fig. 2). This is because hypoxia appears to elicit relaxation of bovine coronary arteries through promoting cytosolic NADPH oxidation, a potential coordinator of multiple mechanisms mediating vascular relaxation (Gupte, et al., 2003; Gupte and Wolin, 2006). In contrast, elevated levels of G6PD and NADPH in bovine pulmonary arteries seems to prevent expression of this hypoxia-elicited NADPH oxidation-mediated relaxation (Gupte, et al., 2005). The elevated levels of G6PD and NADPH in bovine pulmonary arteries have been suggested to be responsible for elevated levels of ROS seen in this vascular segment, because bovine coronary and pulmonary arteries seem to have similar levels of Nox oxidase expression and activity. Bovine pulmonary arteries appear to be partially relaxed by endogenous peroxide under aerobic conditions, and hypoxia seems to promote contraction by removing this basal peroxide-mediated relaxation (Ahmad, et al., 2009, 2010; Burke-Wolin and Wolin, 1989). A reversal of the NADPH oxidation mechanism could also be a contributing factor in the hypoxia-elicited contractile response of rat and bovine pulmonary arteries, because hypoxia has been observed to increase NADPH in these vascular segments (Gupte, et al., 2006, 2010). It appears that Cu,Zn-SOD activity is needed for the generation of peroxide in amounts that maintain a tonic relaxation of bovine pulmonary arteries, because inactivating Cu,Zn-SOD reproduces the effect of hypoxia under aerobic conditions, associated with a marked decrease in the detection of basal peroxide levels (Ahmad, et al., 2009, 2010). Thus, as hypothesized in the model shown in Fig. 2, peroxide generation is evidently a key factor needed for the detection of hypoxia-elicited contraction of bovine pulmonary arteries.

The stimulation of sGC by peroxide metabolism via catalase appears to be a contributing factor to the relaxation of bovine pulmonary arteries that is removed by hypoxia because hypoxia decreases tissue cGMP levels and inactivation of catalase with 3-aminotriazole appears to partially reproduce the effects of hypoxia (Burke-Wolin and Wolin, 1989). While much evidence generally from rat pulmonary arterial smooth muscle preparations support mitochondria as a source of ROS mediating vascular responses to hypoxia (Moudgil, et al., 2005; Waypa and Schumacker, 2005), our studies with methods including siRNA depletion of Nox oxidases has detected evidence for Nox4 as the source of superoxide-derived peroxide removed by hypoxia in bovine pulmonary arteries (Ahmad, et al., 2010; Wolin, et al., 2005). Increasing Nox4 expression by 48 hr organ culture with TGFβ1 also increased the magnitude of the basal relaxation which is removed by hypoxia. In addition, siRNA and pharmacological inhibition studies also confirmed previous evidence (Archer, et al., 1999) from mice deficient in Nox2 suggesting this oxidase does not influence the hypoxic pulmonary vasoconstriction response. Based on the absence of effects of mitochondrial electron transport chain inhibitors, we did not detect a role for mitochondrial-derived ROS in the bovine pulmonary arterial contractile response to hypoxia. However, we have detected

evidence for several additional ROS mechanisms influencing the response of pulmonary arteries to hypoxia. A second effect of the basal levels of Nox4-derived peroxide has the characteristics of maintaining a force enhancing response hypothesized to be mediated through Rho kinase, which is not removed by an acute exposure to hypoxia (Ahmad, et al., 2010). In addition, increased expression of extracellular SOD activity in the vessel wall (resulting from inducing heme oxygenase-1 activity) seems to inhibit the contraction of bovine pulmonary arteries to hypoxia through increasing the conversion of an extracellular source of superoxide into amounts of peroxide that can not adequately be removed by hypoxia (Ahmad, et al., 2009). Thus, our published studies provide evidence for a central role for hypoxia causing contraction of bovine pulmonary arteries by lowering endogenous peroxide that appears to be derived primarily from Nox4. In addition, changes in the stimulation of sGC by peroxide metabolism via catalase seem to be a contributing factor in the response that is observed.

Hypoxia appears to promote contraction of bovine pulmonary arteries through processes including decreasing the $NADP^+$/NADPH ratio, lowering endogenous H_2O_2 and suppressing relaxation by sGC/cGMP system. In addition to these processes, our recent studies uncovered evidence for a new mechanism where bovine pulmonary arteries precontracted with 20 mM KCl showed decreased PKG dimerization in arteries exposed to hypoxia (PO_2 ~10 torr) versus BPA contracted under aerobic conditions (21% O_2) (Neo, et al., 2010a). Hypoxia also promoted decreased phosphorylation of vasodilator-stimulated phosphoprotein (VASP) at the serine-239 site known to be mediated by PKG. Treatment of BPA with 1 mM dithiothreitol increased contraction to 20 mM KCl and abolished contraction to hypoxia, under conditions where it prevented PKG dimerization. Interestingly, when serotonin is used as the precontracting agent, we observed increased PKG dimerization under hypoxic conditions in a manner which may be related to its oxidase activation-associated promotion of pulmonary artery smooth muscle remodeling. Thus, as shown in Fig. 2, attenuation of both PKG activation by disulfide-mediated dimerization and sGC/cGMP activation appear to be a contributing factor to the contraction of BPA by hypoxia.

Circumstances that control when the cGMP independent PKG pathway is favored over the classic sGC stimulated cGMP-dependent PKG pathway remain to be determined, as these pathways co-exist in the vascular smooth muscle cell. By altering $NADP^+$/NADPH ratio, NADPH redox might be a potential regulator of disulfide-mediated dimerization of PKG under both normoxic and hypoxic conditions. While the actual processes regulating dimerization under hypoxia are not known, regulation of PKG by this redox mechanism might be one of the factors contributing to the vasoconstriction of pulmonary arteries seen during hypoxia.

Conclusions

Many independently controlled redox processes influence the production of cGMP by sGC through modulating the availability of NO, thiol and heme sites on sGC controlling activation by NO, and the levels of superoxide and peroxide that influence pathways regulating sGC activity. In addition, our recent studies in bovine arteries support hypothesizing that the activities of peroxide metabolizing pathways in vascular smooth muscle potentially determine the balance between sGC stimulation by peroxide and a cGMP-dependent activation of PKG versus a cGMP-independent activation of PKG resulting from a disulfide-mediated subunit dimerization. While the availability of cytosolic NADPH is an important factor in modulating the generation of peroxide by Nox oxidases and its metabolism by glutathione peroxidase and peroxiredoxin enzymes, it also influences the control of protein thiol redox in ways that can be independent of ROS metabolism. This

could enable cytosolic NADPH oxidation to function as an additional mechanism controlling vascular relaxation through activation of PKG. The apparent control of cytosolic NADPH redox and peroxide generation by hypoxia enables the influence of these systems on cGMP-dependent and cGMP-independent pathways regulating PKG activity to function as a signaling mechanism mediating responses to hypoxia. Although our studies in BPA suggest Nox4 seems to have a key role in generating peroxide in amounts that maintain a cGMP/PKG-associated relaxation which is removed by hypoxia, other sources of changes in peroxide metabolism and/or cytosolic NADPH-regulation of protein thiol redox could be participants in vascular oxygen sensing mechanisms mediated through regulation of PKG. While PKG coordinates the regulation of many of the pathways contributing to vascular responses to hypoxia, the role of PKG versus other mechanisms participating in these responses to hypoxia remains to be defined.

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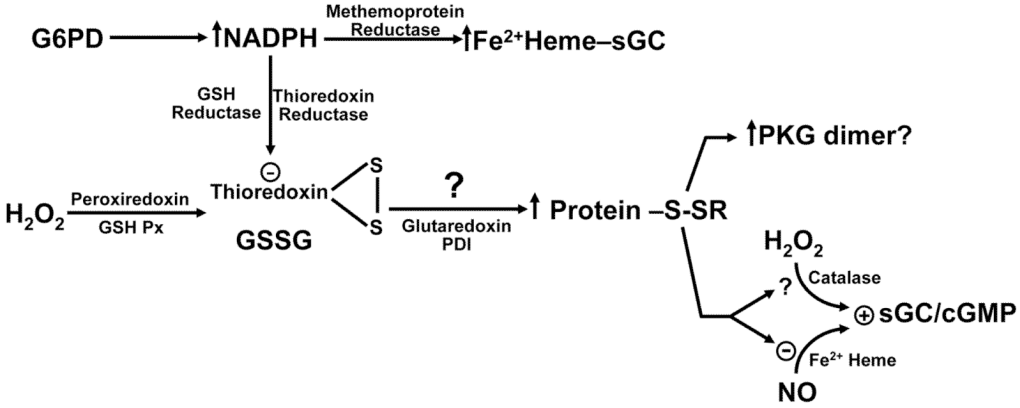

Fig.1.

Model showing how peroxide metabolism and cytosolic NADPH redox controls thiol redox signaling systems, potentially linked to the control of vascular relaxation mediated by activation of soluble guanylate cyclase (sGC) and protein kinase G (PKG).

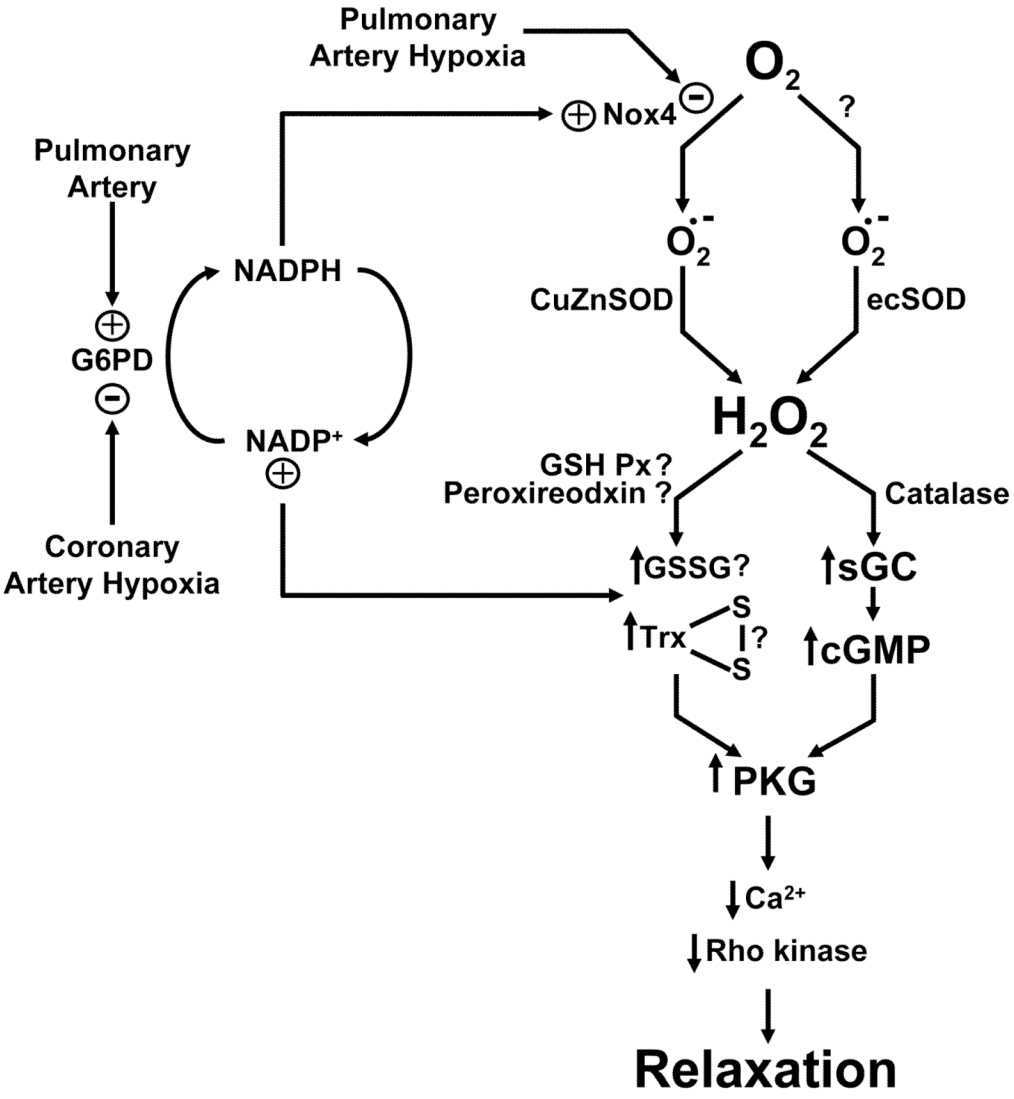

Fig. 2.

Model showing how modulation of peroxide metabolism and cytosolic NADPH redox systems are hypothesized to regulate the balance between sGC-cGMP stimulation of PKG and thiol oxidation-dimerization activation of PKG in the context of their potential participation in the bovine pulmonary artery contractile response to hypoxia. Hypoxia appears to remove a H_2O_2-mediated relaxation, where H_2O_2 originates from SOD metabolizing Nox4 oxidase-derived superoxide ($O_2^{\cdot -}$). This H_2O_2–elicited relaxation may originate from stimulation of PKG activity and/or by hypoxia reversing relaxing mechanisms controlled by cytosolic NADPH oxidation. In this model, the relaxing mechanisms removed by hypoxia potentially involve decreasing intracellular calcium $[Ca^{2+}]_I$ (e.g. through increased uptake by the SERCA pump and decreased Ca^{2+} influx) and suppressing the action of Ca^{2+} on the contractile apparatus through inhibiting Rho kinase activity. The model also includes potential mechanisms through which PKG dimerization could also participate in the cytosolic NADPH oxidation-associated relaxation of bovine coronary arteries to hypoxia. Other sources of superoxide generation may influence hypoxic responses through their conversion to peroxide, including superoxide which is secreted into

the extracellular environment and metabolized by the extracellular form of SOD (ecSOD). GSH Px = glutathione peroxidase.