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Forum Review

Role of Reactive Oxygen Species in Vascular Remodeling Associated with Pulmonary Hypertension

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ABSTRACT

Several manifestations of neonatal pulmonary hypertension are associated with vascular remodeling, resulting in increased muscularity of the small pulmonary arteries. Abnormal structural development of the pulmonary vasculature has been implicated in persistent pulmonary hypertension of the newborn (PPHN). Increased plasma levels of the vasoconstrictor endothelin-1 (ET-1) have been demonstrated in patients with PPHN, which is likely to contribute to hypertension. In addition, several studies have identified a role for ET-1 in the proliferation of vascular smooth muscle cells (SMCs), suggesting that ET-1 may also be involved in the vascular remodeling characteristic of this disease. However, the mechanisms of ET-1-induced SMC proliferation are unclear and appear to differ between cells from different origins within the vasculature. In SMCs isolated from fetal pulmonary arterial cells, ET-1 stimulated proliferation via an induction of reactive species (ROS). Furthermore, other lines of evidence have demonstrated the involvement of ROS in ET-1-stimulated SMC growth, suggesting that ROS may be a common factor in the mechanisms involved. This review discusses the potential roles for ROS in the abnormal pulmonary vascular development characteristic of PPHN, and the treatment strategies arising from our increasing knowledge of the molecular mechanisms involved. *Antioxid. Redox Signal.* 5, 759–769.

PERSISTENT PULMONARY HYPERTENSION OF THE NEWBORN

With the initiation of ventilation and oxygenation at birth, pulmonary vascular resistance decreases and pulmonary blood flow increases. However, in a number of clinical conditions, there is a failure of the pulmonary circulation to undergo the normal transition to postnatal life, resulting in persistent pulmonary hypertension of the newborn (PPHN) (1, 7, 59, 103). In PPHN, pulmonary vascular resistance does not decrease normally at birth, resulting in pulmonary hypertension, right-to-left shunting, and hypoxemia (88). Newborns who die of PPHN exhibit both an increase in the thickness of the smooth muscle layer within small pulmonary arteries and an extension of this muscle to nonmuscular arteries (25). Often, microvascular thrombi occlude these arteries, and there is also proliferation of adventitial tissues (55). These structural changes indicate that *in utero* events have altered the pulmonary circulation. These abnormalities are associated with an increase in the expression of genes that induce vasoconstriction and a reduction in those that induce vasodilation. In particular, there is an inability to regulate properly the production of endothelin-1 (ET-1) such that plasma ET-1 levels are increased (7). Furthermore, there is a down-regulation of genes involved in the production of the vasodilator nitric oxide (NO). The ET-1 and NO cascades are coordinately regulated (46), although the complex mechanisms involved are incompletely understood. In addition, the role of ET-1 and NO in vascular remodeling is unclear.

ENDOTHELIN-1

ET-1, a 21-amino acid polypeptide produced by vascular endothelial cells (ECs), has potent vasoactive properties and is mitogenic for vascular smooth muscle cells (SMCs) (24,

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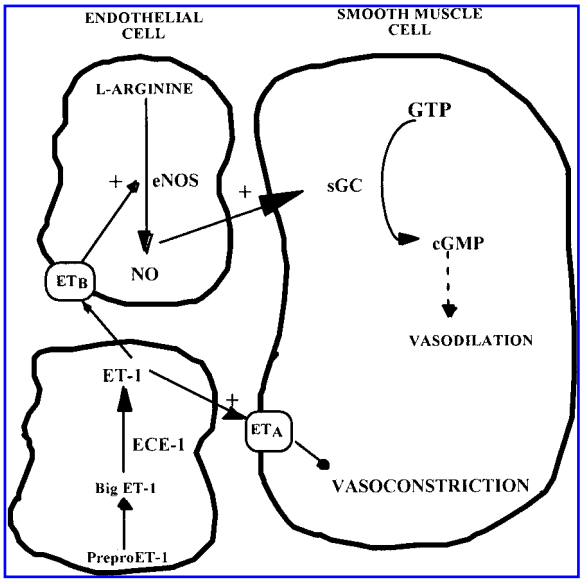

FIG. 1. Regulation of vascular tone by NO and ET-1 signaling in pulmonary arterial endothelial and smooth muscle cells. sGC, soluble guanylyl cyclase.

106). ET-1 is produced by the cleavage of a 203-amino acid precursor (preproET-1) to form proET-1 (Big ET-1). Big ET-1 is then cleaved by endothelin-converting enzyme-1 (ECE-1) into its functional form (95). The complex pulmonary vasoactive effects of ET-1, which may include vasoconstriction and/or vasodilation, are mediated by at least two different receptors: ET_A and ET_B. ET_A receptors, located predominantly on vascular SMCs, mediate vasoconstriction, whereas ET_B receptors, located on vascular ECs, mediate vasodilation (3, 82, 86). Increasing data suggest that NO and ET-1 regulate each other through an autocrine feedback loop (46). For example, stimulation of endothelial nitric oxide synthase (eNOS) activity occurs via ET_B receptor activation, whereas NO-cyclic GMP (cGMP) production increases ET_A receptors in vascular SMCs and inhibits ET-1 secretion and gene expression in vascular ECs (11, 77). Figure 1 illustrates the regulation of vascular tone by the NO and ET-1 signaling cascades. Animal studies suggest that basal ET-1 production has minimal effects on normal fetal, transitional, and postnatal pulmonary vascular tone. However, both animal and human studies suggest that ET-1 plays a significant role in pulmonary vascular pathophysiology.

ET-1-MEDIATED VASCULAR SMC PROLIFERATION IN PPHN

ET_A receptor antagonism attenuates fetal pulmonary hypertension and inhibits the SMC hypertrophy normally associated with ductal ligation (30). This illustrates the important role played by ET-1 in mediating the vascular remodeling characteristic of PPHN. Due to conflicting reports, the role of ET-1 in vascular SMC proliferation remains controversial (26, 32, 34, 84). However, it has been shown that ET-1 has a direct mitogenic effect on pulmonary arterial SMCs isolated from fetal lambs (101). This effect was mediated via an ET_A receptor-induced increase in superoxide production, and was prevented by ET_A receptor blockade or by antioxidant treatment. The pathway likely involves protein G_i, phosphatidylinositol 3-kinase, and NADPH oxidase because pharmacologic inhibitors of these proteins prevented ET-1-induced SMC proliferation. Figure 2 shows the potential signaling pathway for ET-1-mediated proliferation of fetal pulmonary arterial SMCs, and highlights the sites of action of pharmacologic inhibitors.

In pulmonary arterial SMCs isolated from fetal lambs, inhibition of NADPH oxidase reduces the ET-1-mediated mitogenic signal and the increase in reactive oxygen species (ROS) production, indicating that this enzyme lies downstream of the activation of the ET_A receptor (101). Superoxide anion formation has long been known to be vital to the microbicidal activity of phagocytes such as neutrophils, macrophages, and monocytes (18). More recently, it has become apparent that production of ROS also occurs in nonphagocytic cells such as fibroblasts (56), glomerular mesangial cells (71), ECs (51), and SMCs (23, 43). The vascular NADPH oxidase enzyme complex appears to be membrane-associated, catalyzing the one-electron reduction of oxygen using NADPH or NADH as the electron donor. One of the important attributes of the vascular oxidase is that it appears to respond to external signals to generate superoxide. Activation of the oxidase has been demonstrated for angiotensin II (23), serotonin (43), thrombin (67), platelet-derived growth factor (PDGF) (50), tumor necrosis factor-α (56), as well as by biomechanical forces (28). However, it remains unclear as to how this is activation is mediated.

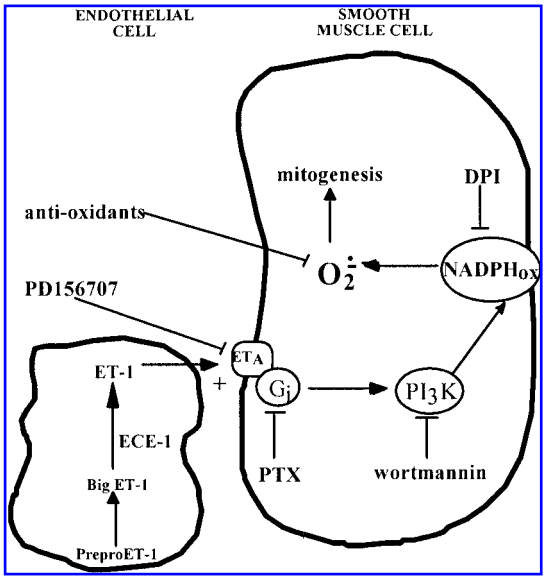

FIG. 2. ET-1-induced proliferation of fetal pulmonary arterial SMCs involves the activation of ET_A receptor, protein G_i, phosphatidylinositol 3-kinase (PI_3K), and NADPH oxidase ($NADPH_{ox}$). Sites of action of the pharmacologic inhibitors PD156707, pertussis toxin (PTX), wortmannin, and diphenyleneiodonium (DPI) are shown.

Treatments that increased cellular levels of ROS also stimulated proliferation in pulmonary arterial SMCs isolated from fetal lambs (101). This is in agreement with other studies in which the addition of exogenous hydrogen peroxide (H_2O_2) or pharmacologic agents that can increase ROS generation appear to stimulate the activation of mitogen-activated protein (MAP) kinases and stimulate cell growth (6, 74, 75, 91). Further studies have demonstrated that SMCs prepared from the systemic circulation can respond to exogenous growth factor stimulation by increasing intracellular production of ROS. For example, PDGF stimulates the production of H_2O_2 in vascular SMCs and leads to SMC growth (91). Conversely, if the PDGF-stimulated rise in H_2O_2 is prevented, the proliferative response to PDGF is blunted (91). Similarly, thrombin stimulates both superoxide and H_2O_2 production in SMCs (67). As with PDGF, treatment with catalase or superoxide dismutase (SOD) to reduce the levels of ROS inhibits thrombin-induced proliferation (67). A recent study demonstrated ET-1-induced activation of MAP kinases in rat aortic SMCs (40). Furthermore, antioxidants and an inhibitor of NADPH oxidase were found to prevent this activation and attenuated ET-1-induced proliferation (40). Thus, overall the available data suggest that ROS mediate growth factor-induced vascular SMC proliferation by activating molecules that stimulate cell cycle progression.

ABNORMAL REGULATION OF NO AND ET-1 CASCADES IN PPHN

The effects of ET-1-induced vascular remodeling are likely to be compounded by increased pulmonary vasoconstriction in PPHN. In an animal model of PPHN, ligation of the ductus arteriosus is associated with a decrease in eNOS expression (66). In addition, there is an increase in the expression of preproET-1 and a decrease in the expression of ET_B receptor. These results suggest a decrease in NO concentrations, thereby decreasing pulmonary vasodilator activity. Furthermore, increased ET-1 concentration and limited ET_B receptor activation would increase pulmonary vasoconstrictor activity. More recent data obtained by constricting the ductus arteriosus *in utero* demonstrated a 106% increase in plasma ET-1 levels and a concomitant 43% decrease in total NO synthase (NOS) activity (66). ET_A receptor antagonism completely blocked the vasoconstriction and preserved NOS activity. In addition, it has been shown that peroxynitrite, formed in the reaction between superoxide and NO, can nitrate and irreversibly inhibit eNOS (102). This nitration was found to be significantly reduced by ET_A receptor blockade (102). These data taken together suggest that ET-1-ET_A receptor-mediated increases in superoxide production with a resultant increase in SMC proliferation and NOS inhibition, coupled with ET_A receptor-mediated vasoconstriction, may play a significant role in the development of PPHN. Figure 3 illustrates the abnormal regulation of the NO and ET-1 cascades in PPHN relative to the normal regulation of vascular tone demonstrated in Fig. 1.

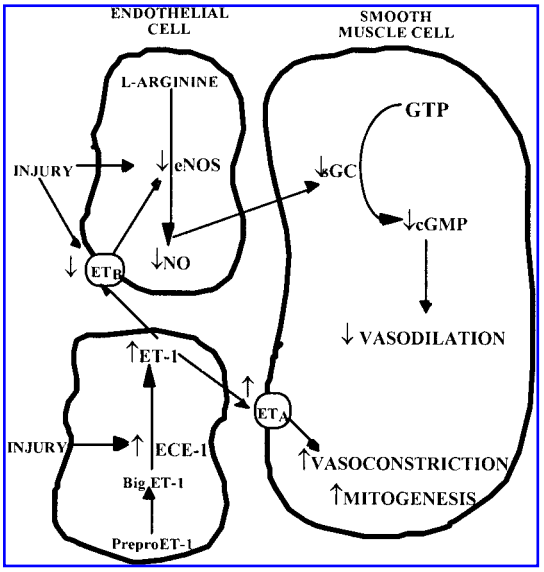

FIG. 3. Abnormal regulation of the NO and ET-1 signaling pathways in PPHN resulting in increased vasoconstriction and SMC proliferation. sGC, soluble guanylyl cyclase.

BIOMECHANICAL FORCES AND ROS PRODUCTION

Fluid shear stress is defined as the tractive force produced by moving a viscous fluid (blood) on a solid body (vessel wall), constraining its motion (62). When ECs are subjected to shear stress, diverse responses are initiated, some of which occur within minutes and others that develop over several hours or days (63). For example, NOS activity, NO production, and eNOS mRNA and protein levels are increased in ECs exposed to shear stress (35, 37, 38, 64, 65, 73, 96, 99). In addition, shear stress stimulates growth factor production by ECs, including basic fibroblast growth factor (bFGF) (56) and PDGF (47, 58). bFGF also up-regulates signaling by vascular endothelial growth factor (VEGF) by elevating levels of the VEGF receptor Flk-1 in vascular ECs (68). Furthermore, shear stress can increase Flk-1 expression via a bFGF-independent pathway (2). Pulsatile blood flow generates mechanical stretch, or cyclic strain, within the vessel walls. Cyclic strain has been shown to increase growth factor production by vascular ECs (78), although the mechanisms involved are unclear. Cyclic stretch has also been demonstrated to induce growth factor expression and signaling in vascular SMCs, including FGF-2 and VEGF (69) and PDGF receptor (93).

ROS play an important role in signal transduction pathways mediated by growth factors, and induce proliferation of vascular SMCs in response to ET-1 (101) and PDGF (50, 91). NADPH oxidase is a major source of ROS in vascular SMCs (23, 101) and ECs (51), and has been shown recently to be involved in VEGF-mediated EC proliferation (98).

Biomechanical forces can also induce ROS production by mechanisms that may be independent of growth factors. Oscillatory shear stress increased NADH oxidase activity and

superoxide production in human umbilical vein endothelial cells (HUVECs) after 1 h, with a progressive increase until 24 h (19). In addition, oscillatory shear stress was found to induce a time-dependent increase in the expression of the redox-sensitive gene heme oxygenase-1 (HO-1), which was blocked by antioxidant treatment. In contrast, laminar shear stress generated an increase in NADH oxidase activity and HO-1 expression after 5 h, although levels had returned to baseline by 24 h. Furthermore, laminar, but not oscillatory shear stress increased the expression of the superoxide scavenger Cu/Zn SOD after 24 h, suggesting that this type of biomechanical force can activate mechanisms to compensate for the oxidative stress (19). This finding has important implications for the onset of atherosclerosis, where oscillatory flow is thought to induce lesion formation, whereas unidirectional flow might be protective (36). The mechanisms that regulate these different patterns of gene expression are unknown, but may involve redox-sensitive transcription factors. For example, the human Cu/Zn SOD promoter region contains two putative binding sites for activator protein-1 (AP-1) (33), while studies of the human HO-1 gene have identified potential binding sites for AP-1 and nuclear factor-κB (NFκB) (42). In bovine aortic endothelial cells (BAECs), fluid shear stress activated the redox-sensitive signaling molecule proline-rich tyrosine kinase 2 (PYK2) via a pathway involving ROS-dependent activation of Src (92). Thus ROS may play multiple roles in the response of ECs to shear stress.

Porcine aortic ECs increased ROS production after exposure to cyclic strain via the activation of NADPH oxidase (27). Cyclic strain also increased NADPH oxidase activity and ROS production in HUVECs; this was associated with an increase in the expression of p22phox (53), a critical subunit of vascular NADPH oxidase (97). Furthermore, cyclic strain increased the activity of NFκB, which was abrogated by NADPH oxidase inhibition, indicating a downstream role for this redox-sensitive transcription factor in HUVECs (53). In BAECs, cyclic strain also induced the activation of PYK2 via a pathway involving protein kinase C-mediated NADPH oxidase activation, followed by ROS-dependent phosphorylation of Src (15). Comparisons between the pathways leading to cyclic strain-induced and shear stress-induced (92) activation of PYK2 in BAECs may help to elucidate the mechanisms by which ECs detect multiple types of biomechanical force.

Overall, these data suggest that the type of biomechanical force and the location within the vasculature may influence the downstream effects of ROS. Increased growth factor and/or ROS production within the pulmonary vasculature in response to biomechanical forces may lead to smooth muscle proliferation via similar mechanisms to those in PPHN.

ABNORMAL REGULATION OF ET-1 AND NO IN PULMONARY HYPERTENSION SECONDARY TO INCREASED PULMONARY BLOOD FLOW

Children with certain congenital heart defects exhibit an increase in pulmonary blood flow, which is associated with vascular remodeling. Although little is known about the mechanisms involved, it is possible that the increased shear stress within the pulmonary vasculature elevates ROS levels via the pathways discussed above. This increase in ROS may then lead to SMC proliferation via mechanisms similar to those evident in PPHN. Pulmonary morphometric analysis of the lungs of children with congenital heart defects shows altered pulmonary vascular growth and remodeling correlating with the child's hemodynamic state (70). These changes are characterized by abnormal extension of muscle into small peripheral arteries and a mild medial hypertrophy of normally muscular arteries, more severe medial hypertrophy of normally muscular arteries, and reduced arterial number and concentration. Several studies demonstrate increased ET-1 plasma concentrations in children with congenital heart disease associated with increased pulmonary blood flow and pulmonary hypertension (100, 105, 107). Recently, a model that mimics a congenital heart defect with increased pulmonary blood flow was established in the lamb with *in utero* placement of an aorta-to-pulmonary artery vascular graft (76). This model is associated with increased pulmonary blood flow and pressure. At 4 weeks of age, these "shunt" lambs have clinical and pathologic sequelae similar to children with congenital heart defects associated with increased pulmonary blood flow and pulmonary hypertension. In shunt animals, plasma ET-1 concentrations were found to be higher (104), and levels of ECE-1 mRNA and protein were elevated in peripheral lung tissue relative to age-matched controls (10). Furthermore, ET_A receptor mRNA and protein levels were increased, whereas ET_B receptor mRNA and protein levels were decreased, in peripheral lung tissue from the shunts relative to controls (10). The predicted result of these gene alterations is increased production of ET-1, increased ET-1-mediated pulmonary vasoconstriction, and decreased ET-1-mediated vasodilation. Shunt lambs also exhibit physiologic alterations in the NO-cGMP cascade, including a selective impairment of endothelium-dependent pulmonary vasodilation. This is suggestive of decreased NO activity because the endothelium-dependent pulmonary vasodilating effects of acetylcholine and ATP were attenuated compared with those in control lambs (76). Using isolated pulmonary arteries, it was found that removal of superoxide enhanced endothelium-dependent relaxation in shunt vessels (89). Thus, the endothelial dysfunction associated with pulmonary hypertension may be due, in part, to excessive superoxide production. This may be linked to increased ET-1 signaling, although additional studies will be required to determine if this is so. Despite decreased endothelial activity in the shunt model, expression of eNOS is elevated (8). Shear stress-induced eNOS transcription, arising from increased pulmonary blood flow, may be involved. However, excessive levels of superoxide present in this model are predicted to inhibit NOS activity (85) and NO bioactivity (87).

A potential role for ROS in the vascular remodeling seen in the shunt model remains to be identified. Several growth factors have been shown to activate NADPH oxidase, including angiotensin II (23) and PDGF (50). Activation of NADPH oxidase by serotonin stimulates the proliferation of bovine pulmonary arterial SMCs via a pathway involving the MAP kinases ERK1/2 (43, 44). Furthermore, ET-1 stimulates the proliferation of SMCs isolated from the pulmonary arteries of fetal lambs via NADPH oxidase-catalyzed ROS production (101). However, a link between increased pulmonary blood flow, el-

evated ET-1 signaling, increased ROS production, and SMC proliferation has not yet been established in the shunt model. Treatment of SMCs isolated from the pulmonary arteries of age-matched animals may reveal if ET-1, or other growth factors, can stimulate SMC proliferation via increased ROS production.

ROLE OF ROS IN ET-1 REGULATION

Several lines of evidence suggest that ROS can regulate cellular levels of ET-1 and mediate its secretion. ET-1 release was increased by HUVECs exposed to cyclic strain, which was blocked by pretreatment with antioxidants (16). These authors then showed an increase in ET-1 promoter activity in BAECs exposed to cyclic strain, which was also blocked by pretreatment with antioxidants. Further studies suggested a role for the transcription factor complex AP-1 in the ROS-mediated increase in ET-1 promoter activity in BAECs (16). Interestingly, ET-1 was found to increase AP-1 DNA binding in rat aortic SMCs via a pathway involving ROS (40). Furthermore, ET-1 stimulated AP-1 activation in rat SMCs via ET_A receptor binding and ROS production (22). This raises the possibility that abnormal regulation of ET-1 expression in PPHN may involve a positive feedback loop; ET_A receptor-mediated increases in superoxide production from SMCs may result in increased ET-1 promoter activity and secretion in the adjacent ECs. In the ductal ligation model of PPHN, levels of preproET-1 and plasma ET-1 were elevated (66). However, additional studies are required to determine if ROS regulate ET-1 expression and release in fetal pulmonary arterial ECs. Furthermore, data obtained from different cell types must be viewed with caution. For example, ROS increased, whereas ROS scavengers decreased, ET-1 release by human mesangial cells (29). Conversely, superoxide has been shown to inhibit ECE-1 activity and decrease ET-1 levels in adult cardiomyocytes (45).

ROLE OF ROS IN NO REGULATION

ROS can influence the bioavailability of NO via several different mechanisms. Superoxide reacts rapidly with NO to form peroxynitrite. Thus, increased ET-1-induced superoxide production in PPHN could potentially reduce even further the levels of bioactive NO, thereby increasing vasoconstriction. The role of NO in SMC growth has not been determined, but it is possible that NO prevents excessive proliferation by regulating superoxide levels. Thus, the ET-1 mediated proliferation of SMCs in PPHN may be compounded by reduced bioavailability of NO.

ROS appear to be involved in the regulation of eNOS gene expression, although the mechanisms involved are unclear. In one study, it was found that antioxidants increased transcription of the eNOS gene in BAECs (72). However, these investigators later demonstrated that H_2O_2, a prooxidant, also increased eNOS promoter activity, as well as eNOS mRNA stability in the same cell type (20). Analysis of the human eNOS promoter sequence has identified potential binding sites for several redox-sensitive transcription factors, including AP-1 and NFκB (49). However, the possibility of redox regulation of eNOS gene expression by these factors, and in particular in pulmonary ECs in the fetal state, has not yet been investigated.

Peroxynitrite has been shown to inhibit the activity of purified eNOS protein (102), presumably by nitration of critical tyrosine residues. Increased levels of peroxynitrite and nitrated proteins have not yet been demonstrated in PPHN, but this may represent one mechanism of eNOS enzyme inhibition in this disease. However, the preservation of NOS activity in ductal ligation lambs infused with an ET_A receptor antagonist (66) suggests that ET-1-induced superoxide production exerts a significant effect on eNOS in PPHN. Furthermore, increased superoxide levels result in the inhibition of eNOS in ECs exposed to NO donors (85). This inhibition was reduced in cells overexpressing SOD (12), illustrating the role for superoxide. NO-mediated eNOS inhibition and the implications for inhaled NO therapy are discussed below.

INHALED NO THERAPY

Exogenously administered inhaled NO is currently utilized as an adjuvant therapy for a number of pulmonary hypertensive disorders, including infants with PPHN. In both animal and human studies, inhaled NO (5–80 ppm) induces rapid and selective pulmonary vasodilation (4, 48, 79, 80). When administered into the airways in gaseous form, NO diffuses into pulmonary vascular SMCs where it increases cGMP levels, causing potent pulmonary vasodilation. No systemic vasodilation occurs because NO is rapidly inactivated by binding with hemoglobin when it reaches the intravascular space (31). Nonrandomized studies demonstrate that inhaled NO decreases pulmonary vascular resistance (PVR) in patients with congenital heart disease (14, 52, 83). In addition, NO decreases PVR and improves oxygenation in adults and children with acute lung injury, although recent randomized trials suggest that the effect is transient and does not change long-term outcome (4, 80). Similarly, several recent multicentered randomized trials have demonstrated that inhaled NO improves oxygenation and reduces the need for extracorporeal life support in newborns with PPHN (79). Although these preliminary data are encouraging, several concerns regarding the safety of inhaled NO remain. One of the most important issues is the safety of acute NO withdrawal. Several studies have noted a potentially life-threatening increase in PVR on acute withdrawal of inhaled NO (5, 17, 41, 57). This "rebound pulmonary hypertension" is manifested by an increase in PVR, compromised cardiac output, and/or severe hypoxemia Exogenous NO exposure inhibits endogenous eNOS activity (9), suggesting that a transient decrease in endogenous eNOS activity during inhaled NO therapy may be a potential mechanism for rebound pulmonary hypertension. Possible causes of rebound pulmonary hypertension and preventative treatments are discussed below.

NO DONORS

Administration of organic nitrates such as nitroglycerin stimulates vasodilation, although the development of nitrate

tolerance shortly after treatment limits their usefulness. The underlying mechanisms are unclear and are likely to be multifactorial (21). One study demonstrated a twofold increase in vascular superoxide production after 3 days of nitroglycerin treatment (60). Furthermore, tolerance was prevented by cotreatment with SOD, highlighting the role for ROS in this inibition (60). A subsequent study identified a membrane-bound NADH oxidase as a likely source of superoxide production (61), and more recently the same investigators demonstrated a role for ET-1 in the pathway (39). It is likely that the increase in superoxide inactivates the NO released from nitroglycerin by the formation of peroxynitrite, resulting in nitrate tolerance.

ET RECEPTOR ANTAGONISTS

Recently, both combined ET_A and ET_B receptor and selective ET_A receptor antagonists have been developed for potential clinical use. In adults with advanced pulmonary vascular disease, bosentan, a combined ET receptor antagonist, decreases PVR and improves exercise tolerance (81). Randomized trials are currently ongoing. Other potential therapeutic uses for ET receptor antagonists include PPHN and pulmonary hypertension associated with congenital heart disease. For example, ET_A receptor blockade prevents ET-1-induced fetal pulmonary arterial SMC proliferation (101), and has been shown to attenuate the vascular remodeling normally associated with ductal ligation in lambs (30). In addition, ET receptor antagonists induce potent pulmonary vasodilation in a lamb model of congenital heart disease with increased pulmonary blood flow. Unfortunately, human data are currently lacking. Lastly, in a recent study looking at the causes of rebound pulmonary hypertension, plasma ET-1 levels were increased by 119.5% in 4-week-old lambs receiving inhaled NO for 24 h (54). Upon acute withdrawal of NO, PVR increased by 77.8%. In contrast, there was no significant increase in PVR in animals infused with PD156707, an ET_A receptor antagonist (54). ET-1 was found to induce superoxide production in SMCs isolated from these 4-week-old lambs, which, in the presence of the exogenous NO, is likely to form peroxynitrite (102). It was suggested that the peroxynitrite then diffuses into the adjacent ECs where it nitrates and inhibits eNOS protein (102). Nitrated eNOS protein was detected in lung tissue of lambs that received inhaled NO, but was reduced in animals treated with PD156707 (102). Therefore, ET_A receptor antagonism may be beneficial in the prevention of rebound pulmonary hypertension upon acute NO withdrawal. Figure 4 demonstrates one potential mechanism of eNOS inhibition by exogenous NO.

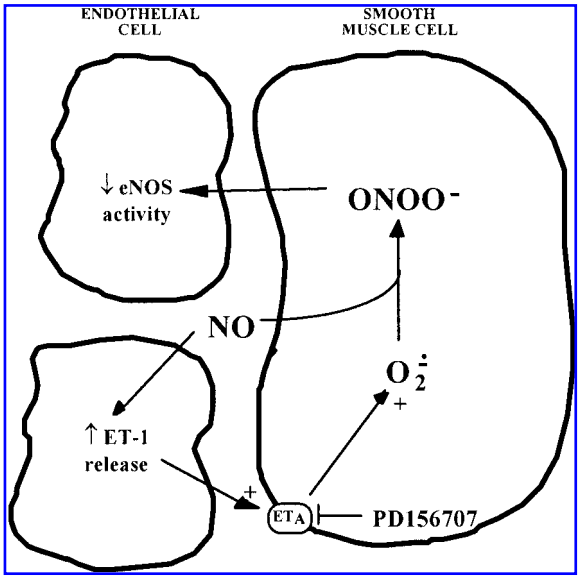

FIG. 4. Potential mechanism of eNOS inhibition by exogenous NO via ET-1-mediated superoxide production and subsequent formation of peroxynitrite ($ONOO^-$).

ANTIOXIDANT THERAPY

ET-1 stimulated fetal pulmonary arterial SMC proliferation via an induction of ROS, which was prevented by treating the cells with ascorbic acid, an antioxidant (101). Higher concentrations of ascorbic acid induced apoptosis in these cells (101). Similarly, antioxidant treatment (94) or overexpression of catalase (13) has been shown to reduce viability and induce apoptosis in other vascular SMCs. Antioxidant treatment may therefore prove useful in the prevention or reversal of ET-1-induced vascular remodeling seen in PPHN. However, the effects of antioxidants on other cell types, especially fetal pulmonary arterial ECs, have yet to be determined. There is currently no available data regarding the effects of antioxidant treatment on human pulmonary hypertension, although the ductal ligation lamb model of PPHN should prove useful in determining the efficacy of this approach.

Antioxidants may also aid inhaled NO therapy by reducing superoxide-mediated peroxynitrite formation. In pulmonary arteries isolated from PPHN lambs, pretreatment with SOD enhanced the relaxation to an NO donor (90). In PPHN animals, treatment with recombinant human Cu/Zn SOD produced selective pulmonary vasodilation. Furthermore, the pulmonary vasodilatory effect of inhaled NO was enhanced in combination with Cu/Zn SOD (90). These events are presumably due to an increased bioavailability of NO, both endogenous and exogenous, by lowering superoxide-mediated peroxynitrite formation. Increased superoxide production mediated by ET-1 (102) and by NO (85) is likely to make a significant contribution to rebound pulmonary hypertension in PPHN patients undergoing inhaled NO therapy, due to peroxynitrite-mediated eNOS inhibition. Therefore, inhaled NO therapy in combination with antioxidant treatment may also help prevent the increase in PVR upon acute NO withdrawal.

CONCLUSION

Relatively little is known about the role of ROS in signaling pathways mediated by other growth factors within the pulmonary circulation. However, it is clear that ROS play a

significant role in the ET-1-mediated vascular remodeling seen in diseases such as PPHN. Further studies are now required to identify downstream targets for these signaling molecules. As ROS apparently play a central role both in vascular remodeling and in endothelial dysfunction in pulmomonary hypertensive disorders, it is likely that antioxidant therapy may represent a useful therapeutic tool. In addition, inhaled NO in conjunction with ET_A receptor antagonists or with antioxidants may prove effective in stimulating pulmonary vasodilation while maintaining normal endothelial function.

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ABBREVIATIONS

AP-1, activator protein-1; BAEC, bovine aortic endothelial cell; bFGF, basic fibroblast growth factor; cGMP, cyclic GMP; EC, endothelial cell; ECE-1, endothelin-converting enzyme-1; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; HO-1, hemo oxygenase-1; H_2O_2, hydrogen peroxide; HUVEC, human umbilical vein endothelial cell; MAP, mitogen-activated protein; NFκB, nuclear factor-κB; NO, nitric oxide; NOS, nitric oxide synthase; PDGF, platelet-derived growth factor; PPHN, persistent pulmonary hypertension of the newborn; PVR, pulmonary vascular resistance; PYK2, proline-rich tyrosine kinase 2; ROS, reactive oxygen species; SMC, smooth muscle cell; SOD, superoxide dismutase; VEGF, vascular endothelial growth factor.

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