Uppregulation of lung soluble guanylate cyclase during chronic hypoxia is prevented by deletion of eNOS

DECHUN LI,1 VICTOR E. LAUBACH,2 AND ROGER A. JOHNS1
1Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287; and 2Department of Surgery, University of Virginia Health System, Charlottesville, Virginia 22908

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Li, Dechun, Victor E. Laubach, and Roger A. Johns. Uppregulation of lung soluble guanylate cyclase during chronic hypoxia is prevented by deletion of eNOS. Am J Physiol Lung Cell Mol Physiol 281: L369–L376, 2001.—Hypoxia upregulates endothelial (e) nitric oxide synthase (NOS), but how eNOS affects soluble guanylate cyclase (sGC) protein expression in hypoxia-induced pulmonary hypertension is unknown. Wild-type (WT), eNOS-deficient [eNOS(-/-)], and inducible NOS (iNOS)-deficient [iNOS(-/-)] mice were used to investigate the effects of lack of NO from different NOS isoforms on sGC activity and protein expression and its relationship to the muscularization of the pulmonary vasculature. After 6 days of hypoxic exposure (10% O2), the ratios of the right ventricle to left ventricle + septum weight (RV/LV+S) and right ventricle weight to body weight, the lung sGC activity, and vascular muscularization were determined, and protein analysis for eNOS, iNOS, and sGC was performed. Results demonstrated that there were significant increases of RV/LV+S in all animals treated with hypoxia. In hypoxic WT and iNOS(-/-) mice, eNOS and sGC α1- and β1-protein increased twofold; cGMP levels and the number of muscularized vessels also increased compared with hypoxic eNOS(-/-) mice. There was a twofold increase of iNOS protein in WT and eNOS(-/-) mice, and the basal iNOS protein concentration was higher in eNOS(-/-) mice than in WT mice. In contrast, the eNOS(-/-) mouse lung showed no eNOS protein expression, lower cGMP concentrations, and no change of sGC protein levels after hypoxic exposure compared with its normoxic controls (P > 0.34). These results suggest that eNOS, but not iNOS, is a major regulator of sGC activity and protein expression in the pulmonary vasculature.

Studies have demonstrated that the nitric oxide (NO)-cGMP signaling pathway participates in the vascular remodeling process in hypoxia-induced pulmonary hypertension (1, 9, 10, 17, 24, 27, 30–32, 34). NO regulates the vascular tone and resistance in the pulmonary circulation and plays an important role in the regulation of vascular smooth muscle cell proliferation, migration, and differentiation in the developing process of pulmonary hypertension both in animals and in humans (11, 18, 20, 35). Congenital disruption of endothelial nitric oxide synthase (eNOS) resulted in mild and persistent systemic and pulmonary hypertension (10, 14, 15, 30, 31). Moreover, in chronic hypoxia-treated rats, there is an upregulation of eNOS and soluble guanylate cyclase (sGC) gene expression, accompanied by increased sGC activity and elevated cGMP levels in the lung (17, 19, 34). However, there is controversy about the role of NO in the development of vascular remodeling in hypoxia-induced pulmonary hypertension. Steudel et al. (30, 31) and Fagan et al. (10) have demonstrated that there are increased pulmonary pressure and resistance and more remodeling in the eNOS-deficient [eNOS(-/-)] mouse compared with the wild-type (WT) mouse after 6 wk of hypoxia exposure, presumably secondary to the reduced production of NO in the vasculature. In contrast, a recent study from our laboratory demonstrated that there is a reduced bromodeoxyuridine labeling index and less muscularization in the pulmonary vasculature in an eNOS(-/-) mouse treated with 4 and 6 days of hypoxia compared with WT animals (25). In addition, the vasodilating effects of NO are hindered in chronic hypoxia-treated animals (6, 28). Inhibition of sGC in isolated rat lungs with chronic hypoxia-induced pulmonary hypertension augmented the pulmonary pressure and resistance (13). These results support the concept that the NO-cGMP signaling pathway modulates pulmonary artery pressure and resistance and participates in the process of vascular remodeling during the development of chronic hypoxia-induced pulmonary hypertension. However, how eNOS expression and how NO derived from different NOS isoforms affects sGC protein expression and modulates its activity in chronic hypoxia-induced pulmonary hypertension are still unknown.

In this study, the protein expression of eNOS, inducible nitric oxide synthase (iNOS), and sGC and enzyme activity of sGC were investigated in chronic hypoxia-treated WT, eNOS(-/-), and iNOS-deficient [iNOS(-/-)] mice, and muscularization of the vasculature (<0.05).
µm in diameter) in these mouse lungs was compared. The results demonstrated that chronic hypoxia induces a parallel upregulation of eNOS and sGC protein expression and increased cGMP levels in WT and iNOS(−/−) mouse lungs but not in eNOS(−/−) mice treated with chronic hypoxia. There is a low level of sGC protein expression and lower cGMP concentrations in eNOS(−/−) mouse lungs compared with WT and iNOS(−/−) mice.

MATERIALS AND METHODS

Animal exposures. WT(+/+) C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME). eNOS(−/−) and iNOS(−/−) mice (10–16 wk old) were generated as previously described (16, 29). All gene-deficient animals used in this study were backcrossed at least seven generations to the parental C57BL/6J strain to minimize the genetic differences between the WT mice and the eNOS(−/−) and iNOS(−/−) mice. The animals were exposed to either normoxia (room air) or hypoxia (10% O₂, normobaric), as previously described (17, 19, 25), for 6 days (n = 6 for each normoxic group and n = 8 for each hypoxic group). Animals were maintained at 22–24°C in a room with a 12:12-h light-dark cycle. All animals were fed standard mouse chow and water ad libitum and were treated humanely in accordance with institutional and federal guidelines.

Tissue collection and weight measurement of right ventricle and left ventricle plus septum. Preliminary hypoxia studies demonstrated that there was an increased eNOS and sGC expression starting at 2 days of hypoxic exposure that reached a peak at 6 days of hypoxia treatment. For the observation of maximal sGC protein expression, we chose to use a 6-day exposure to hypoxia in this study. After 6 days of exposure to either normoxia or hypoxia, the mice were anesthetized with ketamine (100 mg/kg ip), and total body weight was measured with a Mettler AJ100 balance (Mettler Instruments, Hightstown, NJ). Next, the chest cavity was opened, and the lungs were perfused with 2–4 ml of heparinized saline. The lungs and hearts were removed, and the lungs were inflated through the trachea with 4% saline through the pulmonary artery until the lungs appeared white. The lungs and hearts were removed, and the left lungs were slowly inflated through the trachea with 4% paraformaldehyde in PBS (GIBCO BRL, Grand Island, NY) until the edge of the pleura became sharp and were fixed for 4 h before processing for paraffin-embedded sections as previously described (19). The right lung lobe was snap-frozen in liquid nitrogen and stored at −70°C for cGMP measurement and Western blot analysis. The hearts from normoxia- and hypoxia-treated groups were taken, and the right ventricle and left ventricle plus septum were dissected and weighed. The ratio of the right ventricle to the left ventricle + septum weight (RV/LV+S) and the ratio of the right ventricle weight to body weight [RVW (mg/BW (g))] were calculated and are used as an indicator of increased pulmonary pressure and resistance.

Measurement of sGC activity in lung tissue homogenates. sGC activity in the animals treated with 6 days of normoxia or hypoxia was measured as described by Mittal (21). Right lung tissue (20 mg/animal) was homogenized in buffer containing 50 mM Tris-HCl (pH 7.6), 1 mM EDTA, 1 mM dithiothreitol, and 2 mM phenylmethylsulfonyl fluoride. Extracts were centrifuged at 15,000 g for 30 min at 4°C. Protein concentrations were determined using the Bio-Rad protein assay as described by Bradford (3) and others (19). Supernatants containing 50 µg of protein were incubated for 10 min at 37°C in a reaction mixture containing 50 mM Tris-HCl (pH 7.5), 4 mM MgCl₂, 0.5 mM IBMX, 7.5 mM creatine phosphate, 0.2 mg/ml creatine phosphokinase, and 1 mM GTP. The reaction was terminated by the addition of HCl to a final concentration of 0.1 N. cGMP in the reaction mixture was measured using an RIA described previously (19). sGC enzyme activity is expressed as picomoles of cGMP produced per minute per milligram of lung protein.

Immunohistochemical staining for muscularization of pulmonary vessels. After deparaffinization, the sections (5 µm) were stained with antibodies specific for von Willebrand factor (VWF, 1:200 dilution, for the staining of endothelium; DAKO, Carpinteria, CA) and smooth muscle (SM) α-actin conjugated with alkaline phosphatase (1:100 dilution, for the staining of smooth muscle cells; Sigma, St. Louis, MO) to differentiate these cell types in the vasculature, as previously described (34). In brief, tissue sections were incubated overnight at 4°C with a rabbit polyclonal antibody for VWF. The sections were subsequently incubated with a goat anti-rabbit antibody labeled with horseradish peroxidase (HRP; Bio-Rad, Hercules, CA) and anti-SM α-actin conjugated with alkaline phosphatase (1:100). Vascular staining was visualized using the 3’-diaminobenzidine substrate kit (Vector, Burlingame, CA), which produces a brown/black color, whereas SM α-actin staining was accomplished using the new fuchsin (DAKO) substrate to produce a red-colored precipitate in the smooth muscle cells. All of the substrate incubation was controlled at 6 min, and the sections were then counterstained briefly with methyl green or hematoxylin before being mounted with Permount (Fisher, Pittsburgh, PA).

Vessel morphometry. All analysis of vessel morphometry was performed using a blind code. Because the pulmonary resistance mainly comes from the small resistance vessels, the degree of muscularization of small (<80 µm in diameter) vessels was determined in a minimum of 80–160 small vessels/animal lung. Vessels were classified as nonmuscular (NM), partly muscular (PM), or fully muscular (FM) by SM α-actin staining. Nonmuscularized vessels were those that only showed positive staining for VWF (i.e., only stained the endothelial cells and no staining for SM α-actin was apparent in the vessels). Partially muscularized vessels were defined as those exhibiting at least one smooth muscle cell but no continuous media. Fully muscularized vessels displayed a complete continuous smooth muscle media that formed a circular ring. Total muscularized vessels were obtained by the addition of the numbers of partly or fully muscularized vessels classified by the criteria described above. The NM, PM, and FM vessel numbers were expressed as a percentage of the total vessel numbers counted from the section. Computer-assisted image analysis software and Image-Pro software (Media Cybernetics, Silver Spring, MD) were used to assist the morphometric measurements of the vessel sizes.

Western blot analysis for eNOS and sGC protein. The details of Western blotting analysis have been published previously (19). Briefly, the right lobe from each mouse lung was homogenized in homogenization buffer and centrifuged, and the protein content was analyzed by the method of Bradford (3). Protein (100 µg) from the mouse lung was electrophoresed in a 7.5% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane (Bio-Rad). The blots were incubated with a monoclonal anti-eNOS or anti-iNOS antibody (1:1,000 dilution for each antibody; Transduction Laboratories, Lexington, KY) or polyclonal sGC primary antibody (1:500 dilution; Cayman Chemicals, Ann Arbor, MI) followed by a goat anti-mouse or anti-rabbit HRP-labeled secondary antibody (Bio-Rad). eNOS, iNOS, and sGC protein signals were detected using an enhanced chemiluminescence reagent (ECL; Amersham). Blots were exposed to film, and the bands were quantitated using the NIH Image software.
hypoxia exposure. Staining with VWF/SM revealed that there were increased numbers of muscularized vessels (FM and PM) in eNOS(−/−) mice treated with hypoxia compared with normoxic controls. However, in eNOS(−/−) mice, there was a smaller degree of increase in muscularized vessels (FM + PM) in eNOS(−/−) mice treated with 6 days of hypoxia compared with WT and iNOS(−/−) mice treated with hypoxia (Fig. 4). sGC activity in the mouse lung. Comparison of the sGC activity in the mouse lung homogenates revealed that the cGMP concentrations were increased significantly (~2- to 3-fold higher, P < 0.05) in WT and iNOS(−/−) animals treated with hypoxia compared with their normoxic counterparts. In contrast, in eNOS(−/−) animals, the cGMP concentrations were lower in both normoxic and hypoxic groups. In addition, the normoxic eNOS(−/−) animals showed lower cGMP concentrations compared with WT and iNOS(−/−) mice treated with hypoxia (Fig. 5).

Western blot analysis for eNOS, iNOS, and sGC protein. Lung homogenates obtained from normoxic and hypoxia-treated mouse lungs demonstrated that there was an increase of eNOS protein at 6 days in hypoxia-treated WT and iNOS(−/−) mice. There was no eNOS protein detected in either normoxia- or hypoxia-treated eNOS(−/−) mice (Fig. 6). There was no iNOS protein found in the iNOS(−/−) mice, and iNOS protein was increased in both WT and eNOS(−/−) mice treated with hypoxia. Interestingly, the basal iNOS protein levels were higher in eNOS(−/−) mice than in WT mice (Fig. 7). Moreover, there was a twofold increase of sGC α1- and β1-subunit protein that parallels the increase of eNOS protein in WT and iNOS(−/−) mice after exposure to 6 days of hypoxia (P < 0.05, 1-way ANOVA). However, in hypoxic eNOS(−/−) mice, there was an increase of eNOS protein at 6 days in hypoxia compared with normoxic controls (Fig. 1). WT animals showed the largest increase in RV weight in eNOS(−/−) mice treated with hypoxia compared with WT and iNOS(−/−) mice. *P < 0.05 compared with all normoxic and eNOS(−/−) hypoxic groups.

**RESULTS**

**RV/LV+S and RVW/BW.** The ratios RV/LV+S and RVW (mg)/BW (g) were measured from the groups of WT, eNOS(−/−), and iNOS(−/−) mice treated with 6 days of normoxia or hypoxia. In the hypoxia-treated mice, there was a significant increase in RV/LV+S in WT, eNOS(−/−), and iNOS(−/−) mice compared with their normoxic counterparts (P < 0.05). Both hypoxic eNOS(−/−) and iNOS(−/−) mice showed the smallest increase in the ratio of RV/LV+S compared with WT mice (P < 0.05, Fig. 1). WT animals showed the largest increase in the RV/LV+S compared with eNOS(−/−) and iNOS(−/−) mice (P < 0.05). In contrast, RVW (mg)/BW (g) was increased more significantly in both WT and iNOS(−/−) mice than in eNOS(−/−) mice. Comparison of RVW/BW showed that there was no difference between WT and iNOS(−/−) mice (P = 0.78). In contrast, there was a lower right ventricle weight in eNOS(−/−) mice exposed to hypoxia compared with WT and iNOS(−/−) mice [P < 0.005 compared with WT and iNOS(−/−) mice treated with hypoxia, Fig. 2].

**Morphological changes of vasculature after chronic hypoxia exposure.** Staining with VWF/SM α-actin revealed that there were increased numbers of muscularized vessels in WT and iNOS(−/−) mouse lungs compared with normoxic groups (Fig. 3). Morphometric analysis of the pulmonary small vessels (<80 μm in diameter) demonstrated that there was a significant increase for both FM and PM vessel numbers in WT and iNOS(−/−) mice treated with hypoxia compared with normoxic controls. However, in eNOS(−/−) mice, there was a smaller degree of increase in muscularized vessels (FM + PM) in eNOS(−/−) mice treated with 6 days of hypoxia compared with WT and iNOS(−/−) mice treated with hypoxia (Fig. 4).

**Fig. 2.** Ratio of right ventricle (RV) weight (mg) to body weight (g) in WT, iNOS(−/−), and eNOS(−/−) mice. There was a lesser degree of increase in RV weight in eNOS(−/−) mice treated with hypoxia compared with WT and iNOS(−/−) mice. *P < 0.05 compared with eNOS(−/−) normoxic group. **P < 0.005 compared with all normoxic and eNOS(−/−) hypoxic groups.

**Fig. 1.** Ratio of right ventricle to left ventricle + septum weight (RV/LV+S) in wild-type (WT), inducible nitric oxide synthase (iNOS)-deficient (iNOS(−/−)), and endothelial nitric oxide synthase (eNOS)-deficient (eNOS(−/−)) mice treated with 6 days of normoxia (open bars, n = 6 for each group) and hypoxia (solid bars, n = 8 for each group). There was a significant increase in right ventricle weight in all hypoxia-treated animals. *P < 0.05 compared with all normoxic, iNOS(−/−), and eNOS(−/−) hypoxic groups. *P < 0.05 compared with normoxic groups.
Fig. 3. Immunohistochemical staining of smooth muscle α-actin in WT (A and B), iNOS(−/−) (C and D), and eNOS(−/−) mice (E and F). There was increased smooth muscle in hypoxia-treated animals (B, D, and F) compared with normoxic controls (A, C, and E). Original magnification, ×250.

Fig. 4. Muscularization of pulmonary vessels (<80 μm in diameter, fully muscular + partly muscular) in WT, iNOS(−/−), and eNOS(−/−) mouse lungs after exposure to 6 days of hypoxia. There was a smaller increase in muscularized vessels in eNOS(−/−) mice compared with WT and iNOS(−/−) animals. *P < 0.05 compared with normoxic groups. #P = 0.56 compared with all normoxic groups.

Fig. 5. cGMP levels in mouse lungs treated with normoxia or hypoxia. There was a significant increase of cGMP levels in WT and iNOS(−/−) mouse lungs treated with hypoxia compared with normoxia-treated animals. There were low cGMP concentrations in eNOS(−/−) mice treated with normoxia or hypoxia. *P < 0.05 compared with eNOS(−/−) normoxic group. #P < 0.02 compared with all normoxic and eNOS(−/−) hypoxic groups.
the sGC protein showed no change compared with normoxic controls (Fig. 8).

DISCUSSION

Previous studies have demonstrated upregulation of eNOS, iNOS, and sGC protein and mRNA in the lungs from chronic hypoxia-treated rats and mice (19, 24, 34, 36). Congenital disruption of eNOS or inhibition of NO resulted in hypertension in both the systemic and pulmonary circulation, indicating the general vasodilatory role of eNOS and its dependence on the NO levels, the activity of sGC, and integrity of the NO-cGMP signaling pathway. However, the underlying molecular and cellular mechanisms of hypoxia-induced pulmonary vascular remodeling are still unknown. The use of eNOS(−/−), iNOS(−/−), and WT mice enabled us to explore the sGC protein expression under conditions of altered NO production from different sources. The most interesting finding in this study is that congenital disruption of eNOS was associated with unchanged sGC protein production and low sGC activity in the lung in normoxic conditions and during the development of hypoxia-induced pulmonary hypertension. In WT and iNOS(−/−) mice treated with chronic hypoxia, the protein levels for both sGC α1- and β1-subunits and cGMP levels are increased in parallel with the increase of eNOS protein. However, in iNOS(−/−) mice, the basal protein levels of sGC α1- and β1-subunits are higher than in WT mice, and the sGC activity as indicated by cGMP concentrations is the same as in WT mice. This may indicate that the sGC in iNOS(−/−) mouse lung is in a low activity state. In contrast, in eNOS(−/−) mice, the expression of sGC protein and the sGC activity, as indicated in cGMP levels, were both unchanged in chronic hypoxia-treated animals. Interestingly, there was a lesser degree of increase in vascular muscularization that correlated with the smaller increase of RVW/BW in eNOS(−/−) mice treated with hypoxia compared with WT and iNOS(−/−) animals. These results suggest that eNOS or NO produced by eNOS is the main regulator of sGC activity and protein expression. Furthermore, without NO produced by eNOS, the sGC activity in the pulmonary vasculature was lower. In contrast, iNOS, even though its protein expression is increased in eNOS(−/−) mice during chronic hypoxic exposure, plays little or no role in the regulation of sGC protein expression and its activity in hypoxia-induced pulmonary hypertension. It is worthy to note that the spatial relationship of endogenous NO derived from eNOS in endothelial cells may be more efficient to stimulate sGC activity and regulate sGC expression in smooth
muscle cells in the vasculature than NO derived from iNOS in other cell types in hypoxic conditions. This is consistent with the work from Fagan and coworkers (11) who demonstrated that eNOS is the major modulator of the pulmonary vascular tone after chronic hypoxia and that iNOS only played a minor role in the regulation of pulmonary vasoactivity under these conditions.

The role of sGC in the regulation of pulmonary vasoactivity is controversial. Steudel et al. (30) reported that the pulmonary circulation of eNOS(-/-) mice had impaired vasodilation to the NO donor sodium nitroprusside and to inhaled NO in vivo but was intact in vitro when rings were preconstricted with 5-hydroxytryptamine. They concluded that there might be a defect in smooth muscle sGC activity in the pulmonary resistance bed of eNOS(-/-) mice or that NO had little effect on the resting pulmonary tone. Our current results provided experimental evidence for their speculations. In contrast, Fagan and coworkers (11) reported that bradykinin did not reduce pulmonary perfusion pressure in eNOS(-/-) mice and that the NO donor NONOate has the same vasodilatory effects on WT, iNOS(-/-), and eNOS(-/-) mice in the lung. They concluded that sGC function and other NO- and cGMP-stimulated pathways are preserved in the pulmonary resistance arteries of eNOS(-/-) mice. In addition, they found that the response to inhaled NO in eNOS(-/-) mice remains intact, consistent with preserved downstream signal transduction in vivo (10).

In the present study, our data demonstrated that there are no sGC protein quantity differences between normoxic WT and normoxic eNOS(-/-) mice, indicating that the constitutively expressed sGC protein is regulated by unknown factors rather than NO and downstream components of the NO-cGMP signaling pathway are preserved. However, without eNOS-derived NO, the constitutively expressed sGC in the smooth muscle was in a low activity condition and failed to upregulate in hypoxic conditions. In either case, the smooth muscle could not counterbalance the increased vasoconstrictive activity inflicted by hypoxia exposure. A recent study reported by Brandes and coworkers (4) demonstrated that there is increased nitrovasodilator sensitivity in eNOS(-/-) mice. They also found that basal cGMP levels in aortic rings were significantly lower (50 times) in eNOS(-/-) mice than in WT mice. Sodium nitroprusside induced a significant cGMP accumulation in eNOS(-/-) mice compared with WT mice. In addition, they found that the aortic expression of the sGC α1- and β1-subunits in WT and eNOS(-/-) was identical as determined by Western blot analysis (4). They concluded that chronic deficiency of NO in eNOS(-/-) mice restores the NO sensitivity of sGC and enhances vascular smooth muscle relaxation in response to nitrovasodilator agents but did not change its expression. Their results may explain the observations of Fagan et al. (11) and are in agreement with our results in the normoxic eNOS(-/-) mouse lung.

The exact mechanism of how sGC gene expression is affected by NO is not clear. There is evidence from studies in cultured smooth muscle cells that sGC expression is downregulated by NO donors (12, 23). These reports have been used to at least partially explain NO tolerance and the protective role of auto-regulation of cGMP production, particularly in septic shock conditions. In contrast, Black and coworkers (2) demonstrated that there was a coordinated regulation of genes of the NO and endothelin pathways during the development of pulmonary hypertension in fetal lambs (22). They found that ligation of the ductus arteriosus in utero in lambs was associated with decreased lung expression of eNOS mRNA and protein. There was also decreased expression of sGC α1- and β1-subunit protein and increased expression of cGMP-specific phosphodiesterase V mRNA. These reports supported our conclusion that eNOS-produced NO may be the main regulator or stimulator for sGC protein expression in hypoxia-induced pulmonary hypertension, and the lack of NO-stimulated sGC production in the pulmonary vasculature may be one of the mechanistic reasons for the increased pulmonary pressure in
eNOS(+/−) mice as already demonstrated in eNOS(−/−) mouse aorta (4). The NO-cGMP signaling pathway may have an autoregulatory mechanism to control its downstream component gene expression and enzyme activity to maintain proper vascular tone and resistance by means of feedback.

Various studies have provided conflicting data regarding the potential role of NO in pulmonary vascular remodeling induced by hypoxia. It has been demonstrated that hypoxia can upregulate many genes, including growth factors and vasoconstrictors. The increased expression of growth factor and vasoconstrictor may serve as mitogens for the pulmonary arterial smooth muscle and facilitate vascular remodeling (5, 33). In a rat model of chronic hypoxia, upregulation of eNOS in the small vessels of the lung precedes and progresses with the time course of muscularization of these vessels (34, 36). In addition, the sGC expression and enzyme activity showed a remarkable increase in chronic hypoxia-induced pulmonary hypertension in rats. Immunohistochemistry and in situ hybridization demonstrated that the increased sGC protein and mRNA were mainly from newly muscularized pulmonary arteries that are responsible for the increased pulmonary vascular pressure and resistance (19). In this study, we found that, in eNOS(−/−) mice, there was no significant increase in muscularization after 6 days of hypoxia exposure compared with that seen in WT and iNOS(−/−) mice. Furthermore, there was a reduced RVW (mg/BW g) in eNOS(−/−) mice. The reduction of RV/LV+S in eNOS(−/−) mice might be a result of hypertrophy of the left ventricle because of mild systemic hypertension, and this ratio may not be a reliable parameter for the assessment of pulmonary muscularization in eNOS(−/−) mice as in WT animals. However, a lesser degree of increase in the RVW (mg/BW g) might reflect the real status of pulmonary vascular resistance. The decreased RVW (mg/BW g) in eNOS(−/−) mice could be directly related to the lesser degree of muscularization in the pulmonary vasculature. The exact role of how NO affects ventricular hypertrophy is controversial. However, a study reported by Rouet-Benzineb et al. (26) demonstrated that 4 wk of administration of N-nitro-L-arginine methyl ester, a nonspecific NOS inhibitor, to hypoxia-exposed rats attenuated pulmonary arterial pressure and RVW/BW (vs. hypoxia-exposed group). Moreover, a recent study by de Oliveira and coworkers (7) showed that rats with prolonged treatment with a low dose of NOS inhibitor (7.5 mg·kg−1·day−1 for 4–6 mo) developed cardiomyocyte hypertrophy rather than hypertrophy. Therefore, it appears that the process of myocyte hypertrophy depends to some extent on NO formation within the heart during pressure overload. They proposed the following two possible explanations: the first was based on a systemic deficiency of NO, leading to a decrease in blood supply to the heart muscle, and the other was based on a local deficiency of NO, causing metabolic changes in the cardiomyocyte itself. Further studies are needed for the elucidation of the role of NO in ventricular hypertrophy. Several reports have found that there were no structurally significant differences in the vessel morphology in WT and eNOS(−/−) mouse lung in normoxic conditions (10, 30, 31). The increased pulmonary resistance and pressure in eNOS(−/−) mice might be functional and may be a result of the reduction of NO derived from eNOS and low activity of sGC.

In summary, our studies demonstrate that eNOS(−/−) mice treated with hypoxia have low levels of cGMP and sGC protein compared with WT and iNOS(−/−) mice and less remodeling in the pulmonary vasculature. We conclude that eNOS-produced NO is the main activator or stimulator of sGC in the pulmonary vasculature. The lower levels of sGC activity in the eNOS(−/−) mouse lung may be one of the mechanistic reasons accounting for the increased pulmonary resistance and pressure. Selectively activating sGC activity and increased cGMP production may be taken as a new strategy for the treatment of pulmonary hypertension in the future.

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