Regulation of NO-elicited pulmonary artery relaxation and guanylate cyclase activation by NADH oxidase and SOD

SACHIN A. GUPTE, TASNEEM RUPAWALLA, KAMAL M. MOHAZZAB-H., AND MICHAEL S. WOLIN
Department of Physiology, New York Medical College, Valhalla, New York 10595

Gupte, Sachin A., Tasneem Rupawalla, Kamal M. Mohazzab-H., and Michael S. Wolin. Regulation of NO-elicited pulmonary artery relaxation and guanylate cyclase activation by NADH oxidase and SOD. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1535–H1542, 1999.—We have previously reported that inhibition of Cu/Zn superoxide dismutase (SOD) in endothelium-removed bovine pulmonary arteries (BPA) attenuates nitrovasodilator-elicited relaxation and that a NADH oxidase linked to the redox status of cytosolic NADH is the major detectable source of superoxide (O2·−) production in this tissue. In the present study, we investigated whether NADH oxidase-derived O2·− participated in inhibition of nitrovasodilator-elicited relaxation and soluble guanylate cyclase (sGC) stimulation. Lactate (10 mM) and pyruvate (10 mM) were employed to increase and decrease, respectively, NADH-dependent O2·− production in the BPA presumably by modulating cytosolic NAD(H) through the lactate dehydrogenase reaction. A 30-min pretreatment with 10 mM diethyldithiocarbamate (DETCA) was used to inhibit Cu/Zn SOD, and 5-nitroso-N-acetylpenicillamine (SNAP) was employed as a source of nitric oxide (NO). Lactate or pyruvate did not alter relaxation to NO. However, when the response to NO was inhibited by DETCA, lactate potentiated and pyruvate reduced the inhibitory effects of DETCA. SOD attenuated the inhibitory effects of DETCA plus lactate. In the presence of 10 µM SNAP, the activity of sGC in a BPA homogenate preparation (which was reconstituted to approximate tissue conditions) was not altered by SOD. However, NADH (0.1 mM) decreased sGC activity by 70% and this effect of NADH was attenuated in the presence of SOD. Thus, cytosolic NADH redox and Cu/Zn SOD activity have important roles in controlling the inhibitory effects of O2·− derived from NADH oxidase on sGC activity and cGMP-mediated relaxation to nitrovasodilators in BPA.

nitric oxide; redox; superoxide anion; superoxide dismutase; reduced nicotinamide adenine dinucleotide

IT IS WELL ESTABLISHED that cGMP derived from cytosolic or soluble guanylate cyclase (sGC) is an important intracellular mediator of vascular smooth muscle relaxation elicited by nitric oxide (NO). The inhibitory effect of superoxide (O2·−) on NO-elicited vascular relaxation was one of the key observations that resulted in the identification of NO as a mediator of endothelium-dependent relaxation (9, 11, 17). Alterations in the interaction of O2·− with NO signaling are now emerging as an important process in the expression of many vascular diseases, including atherosclerosis (20, 24, 27), hypertension (25, 26, 29), and diabetes (7, 8, 23). It was initially demonstrated employing an inhibitor of Cu/Zn superoxide dismutase (SOD) activity that SOD functioned in vascular smooth muscle in a manner that enabled NO to elicit vascular relaxation by preventing its inactivation by endogenous O2·− (5). Release of the endothelium-derived relaxing factor eventually thought to be NO in its vasoactive form was subsequently demonstrated to be dependent on the Cu/Zn SOD activity present in endothelium (18, 21). The effects of an acute inhibition of SOD appear to be selective for relaxing mechanisms involving NO and the stimulation of sGC, since responses to relaxing agents that function through cAMP do not appear to be altered by inhibition of SOD (5, 21). Although the role of SOD in enabling NO to stimulate sGC and produce vascular relaxation is well accepted, processes involved in regulating sources of O2·− production that could potentially attenuate these responses are rather poorly understood.

Examination of the effects of tissue metabolites on the detection of O2·− production by lucigenin chemiluminescence resulted in the observation that lactate appeared to increase the production of this reactive O2·− species (22). This observation resulted in the accumulation of evidence that cytosolic NADH could control the production of O2·− in vascular endothelium (14) and smooth muscle (22) and in the observation that NADH oxidase activity was a prominent source of O2·− production in bovine pulmonary arterial smooth muscle (12, 15, 16). Characterization of the source of this O2·−-producing activity resulted in the identification and characterization of a microsomal NADH oxidase containing a cytochrome b-558 in bovine pulmonary arterial smooth muscle (15). Studies in cultured rabbit aortic smooth muscle have observed that angiotensin II increases NADH oxidase activity and the expression of a key P21phox subunit similar to a component of the phagocyte NADPH oxidase, and this effect of angiotensin II appears to be essential for the growth-promoting actions of this agent (10, 28). Previous work in endothelium-removed bovine pulmonary arteries suggested that changes in PO2 and lactate modulated a cGMP-mediated relaxation response as a result of alterations in NADH oxidase-derived H2O2 (12, 16, 22), because H2O2 is able to stimulate the activity of sGC by a mechanism involving its metabolism by catalase (3, 4). However, the effects of modulation of O2·− derived from NADH oxidase on NO-elicited vascular smooth muscle relaxation and activation of sGC have not been previously examined. We postulated that modulating the amount of O2·− produced by NADH oxidase through altering the redox of its substrate NAD(H) could be an
important mechanism of control of the inhibitory effect of \( \text{O}_2 \) on these NO-elicited responses. Therefore, the purpose of this study was to characterize the effects of metabolic alterations in NADH oxidase-derived \( \text{O}_2 \) on NO-elicited bovine calf pulmonary arterial smooth muscle relaxation and sGC activation.

**MATERIALS AND METHODS**

Materials. GTP, IBMX, phosphocreatine, creatine phosphokinase, N\(^\text{6}\)-nitro-L-arginine (L-NAME), indomethacin, oxyurinol, rotenone, SOD from bovine blood (3,500 U/mg), catalase from Aspergillus niger (6,600 U/mg protein), Tiron, diphenyldiamine (DPD), lactic acid, sodium pyruvate, diethyldithiocarbamic acid (DECTA), collagenase (type 4), soybean trypsin inhibitor (type 1-S), elastase (type VI), EDTA, MOPS, 8-bromo-cGMP, forskolin, NADH, and NAD were purchased from Sigma Chemical (St. Louis, MO). Nitroglycerin solutions were prepared by dissolving 0.4 mg sublingual tablets (Parke-Davis, Morris Plains, NJ) in distilled water. All other chemicals were analyzed reagent grade from Baker Chemical (Phillipsburg, NJ). cGMP enzyme immunoassay kit was purchased from Cayman Chemical (Ann Arbor, MI). S-nitroso-N-acetylpenicillamine (SNAP) was synthesized by methods previously published (5). Lactate solutions (1 M) were prepared by dissolving lactic acid in water followed by adjustment of pH with NaOH (1 M) to 7.4.

Preparation of homogenate fraction of calf pulmonary artery. Bovine lungs were obtained immediately after slaughter and maintained in ice-cold oxygenated saline solution containing (in mM) 125 NaCl, 2.7 KCl, 1.8 CaCl\(_2\), and glucose buffered (pH 7.4) with 23.8 Tris·HCl while being transported from the slaughterhouse to the laboratory (3). The homogenate was prepared by a previously described method (15). In brief, after isolation of the major lobar pulmonary arteries from four animals and removal of the endothelium, the medial layer of the artery was finely minced with a commercial meat grinder and then digested with a collagenase (91 mg/ml) solution containing soybean trypsin inhibitor (0.25 mg/ml) and elastase (0.125 mg/ml) in 20 mM MOPS-KOH buffer (pH 7.4) containing 250 mM sucrose (1 g tissue/2 ml buffer) at 37°C for 15 min. After the addition of glutathione to a final concentration of 10 mM, the tissue was subsequently homogenized at 0–5°C in an Eberbach homogenizer at maximum speed with five 20-s treatments. The material retained on a stainless steel sieve was rehomogenized in 50% of the original volume of MOPS-sucrose buffer. The pooled vessel homogenates were filtered through four layers of cheesecloth, and the homogenate was reconcentrated to approximate tissue enzyme levels in the assay of sGC activity. Briefly, homogenate (15 ml) was reconcentrated eightfold by removing the homogenization buffer using a Ultrafree-45 centrifugal filter having a pore size of 5,000 Da by centrifuging it at 3,000 rpm over a period of 10–12 h at 4°C. It was found that the presence of glutathione was essential for the observation of reproducible effects of probes on the activity of sGC in this homogenate preparation. The reconcentrated homogenate was subsequently diluted twofold into sGC assays.

Determination of sGC activity in homogenate. Guanylate cyclase activity in the arterial homogenate was determined by enzyme immunoassay. Briefly (3), reaction mixture (0.2 ml final volume) contained 20 mM MOPS-KOH (pH 7.4), 0.1 mM GTP, 2 mM MgCl\(_2\), 0.3 mM of the phosphodiesterase inhibitor IBMX, a GTP-regenerating system consisting of 10 mM phosphocreatine and 150 U/ml creatine phosphokinase, 0.1 ml of concentrated homogenate, and test agents, as indicated. Assays of sGC activity were initiated by the addition of arterial protein. Incubations were conducted for 10 min at 37°C, and they were terminated by the addition of 0.1 ml of preheated 12 mM EDTA. This was followed by boiling the assay mixtures for 10–15 min. Each tube was centrifuged at 15,000 rpm, and the supernatant, which was subsequently diluted fivefold, was used to estimate cGMP by enzyme immunoassay. The 10-min incubation for the assay of sGC activity was chosen to optimize the detection of cGMP under the wide variety of conditions examined. In preliminary experiments for the present study and in our previous work, it was confirmed that the activity of sGC in the presence of NO donors including SNAP and \( \text{H}_2\text{O}_2 \) generating systems is linear with time for 10 min. The production of \( \text{O}_2 \) by NADH oxidase activity in the bovine pulmonary artery preparation (in the presence of a NADH-regenerating system) has also been observed to be constant with time (16).

Determination of changes in force in bovine intrapulmonary artery. The second and third branches of the main pulmonary artery were isolated and cut into rings ~4 mm in diameter and width, and the endothelium was removed by gentle rubbing. As previously described (3), the arterial rings were mounted on wire hooks attached to Grass (FT03) force displacement transducers for measurement of changes in isometric force. Tension was adjusted to 5 g, which is the optimal passive force for maximal contraction. Changes in force were recorded on a Grass polygraph (model 7). Vessels were incubated in 10-ml baths (Metro Scientific), which were individually thermostated (37°C) in Krebs buffer gassed with 95% air-5% \( \text{CO}_2 \). Krebs buffer contained the following (in mM) 118 NaCl, 4.7 KCl, 1.5 CaCl\(_2\), 25 NaHCO\(_3\), 1.1 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), and 5.6 glucose. Arteries were incubated for 2 h during which passive tension was adjusted to maintain 5 g. The vessels were then depolarized with Krebs containing KCl (123 mM) in place of NaCl before we conducted the experiments. Pulmonary arterial Cu/Zn SOD was inhibited using the copper chelator DETCA (10 mM). After the vessel was incubated in DETCA for 30 min, it was washed out with Krebs solution several times, and the vessels were incubated for 15–20 min in Krebs solution before contracting again with 40 mM KCl. This concentration of KCl was chosen because it prevented the detection of alterations in force generation by all the combinations of probes employed. The vessels were allowed to stabilize, then they were relaxed with different cGMP-dependent and cAMP-dependent relaxing agents in the absence and presence of probes described in the experiments reported in the RESULTS.

Statistical analysis. All results are expressed as means ± SE of the number (n) of animals employed or determinations made in separate preparations of pooled homogenates derived from arteries of four animals. Comparisons between groups were made with an ANOVA, and a Student’s t-test employing a Bonferroni correction was used to determine statistical significance between groups. \( P < 0.05 \) was considered to be statistically significant.

**RESULTS**

Effects of lactate and inhibition of SOD on NO-elicited relaxation of endothelium-removed pulmonary arteries. To test the hypothesis that NO stimulation of cGMP-mediated vascular relaxation is inhibited by \( \text{O}_2 \) derived from NADH oxidase, a DETCA pretreatment was used to inhibit Cu/Zn SOD (5), and lactate was employed to elevate the production of \( \text{O}_2 \) presumably
by increasing the levels of cytosolic NADH through the lactate dehydrogenase reaction (16, 22). Lactate (10 mM) did not alter the concentration-dependent relaxation of pulmonary arteries to the NO donor SNAP. Whereas, as shown in Fig. 1, the previously reported (5) inhibitory effects of inactivation of endogenous SOD by a 30-min pretreatment with the 10 mM of the copper chelator DETCA on NO-elicited relaxation were potentiated in the presence of 10 mM lactate. Similarly, the relaxation elicited by NO generated as a result of the acute addition of acidified nitrite (pH 2, diluted 1,000-fold to final concentrations of 0.1 mM nitrite) and to nitroglycerin (1 µM) were also attenuated by lactate only in arteries pretreated with DETCA. The relaxation to acidified nitrite and nitroglycerin in DETCA-pretreated arteries of 49.9 ± 5.6 and 58.5 ± 7.9%, respectively, was reduced to 35.2 ± 3.5% (P < 0.05, n = 6) and 43.3 ± 6.6% (P < 0.05, n = 11) in the presence of 10 mM lactate.

Effects of exogenous SOD on the attenuation of NO-elicited relaxation by lactate plus inhibition of endogenous SOD. To examine if O₂⁻ is mediating the inhibitory effects of SOD inhibition plus 10 mM lactate on arterial relaxation to the NO donor, 0.3 µM SOD was added to the tissue bath after the washout of DETCA, before the addition of SNAP. The presence of added SOD markedly prevented inhibitory effects of DETCA pretreatment plus lactate on the relaxation to SNAP (see Fig. 2). The response to SNAP was not significantly altered by the presence of 0.3 µM SOD in the absence of the other probes that alter O₂⁻ metabolism. The presence of the intracellular scavenger of O₂⁻, 10 mM Tiron, also attenuated the effects of DETCA pretreatment plus lactate. For example, relaxation to the 1 µM dose of SNAP of 82.4 ± 6.7% (n = 6) was not altered by 10 mM Tiron in the absence of DETCA pretreatment (87.5 ± 7.1% relaxation), but the attenuation (P < 0.05) of relaxation to SNAP by DETCA pretreatment plus lactate (24.1 ± 6.8% relaxation) was markedly suppressed (P < 0.05) in the presence of Tiron (54.9 ± 6.3% relaxation).

Effects of pyruvate and inhibition of SOD on NO-elicited relaxation. The hypothesized role of NADH oxidase in controlling the attenuation of relaxation to NO by DETCA pretreatment was further examined by employing 10 mM pyruvate as a potential method of lowering cytosolic NADH through the lactate dehydrogenase reaction. The inhibitory effects of inactivation of SOD by DETCA pretreatment on relaxation of pulmonary arteries to SNAP is markedly reduced by the presence of 10 mM pyruvate (see Fig. 3). The data in Fig. 3 also indicate that 10 mM pyruvate does not alter the response to SNAP.

Absence of effects of lactate and inhibition of SOD on relaxation to forskolin and 8-bromo-cGMP. The effects of DETCA pretreatment and the presence of 10 mM lactate on relaxation of endothelium-removed bovine pulmonary arteries to forskolin, an agent thought to function through cAMP, were examined to confirm that these treatments were selective in their inhibitory effects.
effects on relaxation to NO-mediated responses. As shown by the data in Fig. 4, the response to forskolin was not altered by DETCA pretreatment and/or the presence of lactate. The relaxation to 0.3 mM 8-bromo-cGMP of 37.3 ± 6.9% (n = 6) was not altered by DETCA pretreatment (44.3 ± 7.9%), 10 mM lactate (51.1 ± 6.1%), or DETCA plus lactate (51.5 ± 7.8%).

Properties of sGC activity in the homogenate of bovine pulmonary arteries. To examine the potential influence of NADH oxidase activity on sGC in the presence of enzyme levels that approximated the amounts present in the intact vascular tissue, homogenates obtained from the media of the bovine pulmonary artery wall were reconcentrated by ultrafiltration. Interestingly, the properties of sGC activity in the homogenate shown in Fig. 5 suggest that the enzyme was being activated by endogenously formed H$_2$O$_2$. As demonstrated by the data in Fig. 5, sGC activity (control) was inhibited in the presence of 1 µM of a fungal catalase from Aspergillus niger, 0.1 M sodium formate, and 10 µM DPI. It has been previously demonstrated that the stimulation of sGC by H$_2$O$_2$ is attenuated 1) by fungal catalase as a result of it competing with the mammalian form of catalase for the metabolism of H$_2$O$_2$ (3, 6), 2) by formate reducing the levels of the form of catalase (compound I) which is thought to stimulate sGC (3), and 3) by DPI inhibiting the biosynthesis of endogenous O$_2^-$-derived H$_2$O$_2$ (12). On the basis of the absence of effects of inhibitors of xanthine oxidase (0.1 mM oxypurinol), cyclooxygenase (10 µM indomethacin), nitric oxide synthase (100 µM l-NNA), and NADH dehydrogenase of the mitochondrial electron transport chain (50 µM rotenone) on sGC activity in the homogenate (see Table 1), these potential sources of H$_2$O$_2$ production do not appear to be the origin of production of endogenous H$_2$O$_2$ that is stimulating the activity of sGC. The data in Fig. 5 indicate that removal of the activation of sGC by endogenously formed H$_2$O$_2$ is needed to observe the expression of SNAP-elicited NO stimulation of sGC.

Effects of probes for O$_2^-$ derived from NADH oxidase on homogenate sGC activity. Combinations of NAD(H) and SOD were employed to evaluate the effects of O$_2^-$-derived from endogenous NADH oxidase on the activity of sGC in the arterial homogenate. The activity of sGC stimulated by endogenously produced H$_2$O$_2$ (see Fig. 6) or NO derived from 10 µM SNAP (see Fig. 7) was attenuated by 0.1 mM NADH. Although the addition of

### Table 1. Effects of inhibitors of enzymatic sources of production of O$_2^-$ and H$_2$O$_2$ on sGC activity in reconcentrated pulmonary arterial homogenates

<table>
<thead>
<tr>
<th>Condition</th>
<th>sGC Activity, pmol cGMP·min$^{-1}$·mg tissue$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>115 ± 43</td>
</tr>
<tr>
<td>Oxypurinol</td>
<td>72 ± 30</td>
</tr>
<tr>
<td>Nitro-L-arginine</td>
<td>103 ± 22</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>106 ± 41</td>
</tr>
<tr>
<td>Rotenone</td>
<td>96 ± 24</td>
</tr>
</tbody>
</table>

Values are means ± SE of 7 determinations made in each condition.

sGC, soluble guanylate cyclase.
SOD (0.3 µM) did not directly alter sGC activity under these conditions, it reversed the inhibitory effects of NADH. Under the conditions shown in Figs. 6 and 7, Tiron (10 mM) also reversed the inhibitory effects of NADH on sGC activity in the absence (105 ± 43 pmol cGMP·g⁻¹·min⁻¹, n = 6) and presence of 10 µM SNAP (123 ± 50 pmol cGMP·g⁻¹·min⁻¹, n = 6). In contrast to the inhibitory effects of NADH, 0.1 mM NAD (see Figs. 6 and 7) did not alter the stimulation of sGC by H₂O₂ or NO. In addition, NADPH (0.1 mM) or NADP did not inhibit the H₂O₂- or NO-stimulated activity of sGC in arterial homogenates (data not shown).

**DISCUSSION**

Observations made in the present study are consistent with NADH oxidase being a prominent contributor to the inhibitory effects of endogenously produced O₂ on NO-elicited relaxation and sGC activation. In addition, the activity of SOD is a key determinant of the expression of the inhibitory effects of O₂ derived from this system. Figure 8 contains a model for the mechanisms through which the redox systems examined in this study are hypothesized to influence NO-elicited relaxation and sGC activation in bovine pulmonary arterial smooth muscle.

Study of the influence of NADH oxidase on NO stimulation of sGC required characterizing the function of several redox systems present in the reconcentrated homogenate. The initial observation of a minimal stimulation of sGC activity by the NO donor SNAP prompted us to investigate the influence of endogenously produced oxidant species, such as O₂⁻ or H₂O₂. Our previous work on sGC activity in the 20-fold diluted 100,000 g supernatant fraction of bovine pulmonary arterial smooth muscle (3, 4) and with the heme-containing enzyme purified from bovine lungs (6) identified an essential role for peroxide metabolism by catalase in the stimulation of cGMP production and an inhibitory effect of O₂⁻. In these studies, the addition of various mammalian catalase preparations permitted...
the observation of a stimulation of sGC activity by H$_2$O$_2$ produced by the auto-oxidation of redox cofactors or by the addition of enzymatic generating systems. This mechanism of stimulation of sGC was inhibited by a fungal catalase preparation derived from A. niger and by agents such as formate that react with an oxidized species of catalase (compound I) formed during the metabolism of peroxide. Observation in the undiluted homogenate preparation employed in the present study of an absence of restoration NO stimulation of sGC by the addition of catalase and inhibition of cGMP production by antagonists of sGC stimulation by catalase-dependent peroxide metabolism permitted us to realize the importance of endogenous H$_2$O$_2$ production and catalase activity. Thus stimulation of sGC activity by the NO donor SNAP was readily observed in the presence of probes that eliminated the sGC activating effects of H$_2$O$_2$ metabolism by endogenous catalase. In addition, because NO appears to be a very potent inhibitor of both endogenous catalase activity and the cGMP-associated relaxation of bovine pulmonary arteries to H$_2$O$_2$ (13), it is likely in the present study that NO becomes the primary stimulator of sGC activity even under the experimental conditions where endogenously produced H$_2$O$_2$ is also present in the homogenate and intact pulmonary artery.

The function of NADH oxidase in the arterial homogenate preparation appears to have a prominent influence on the expression of sGC activity. In the presence of NADH, but not NAD, the stimulation of sGC activity by either NO or endogenously produced H$_2$O$_2$ is markedly attenuated. Because the addition of SOD prevents expression of the inhibitory effects of NADH on sGC stimulation by NO or H$_2$O$_2$, O$_2$ is the product of the NADH oxidase reaction that is affecting the activity of sGC. Although our data do not identify the exact source of H$_2$O$_2$ production involved in the stimulation of sGC activity in the homogenate preparation, the actions of DPI and the inhibitors reported in Table 1 suggest the involvement of a flavoprotein other than nitric oxide synthase, xanthine oxidase, and mitochondrial NADH dehydrogenase. Thus it appears that the presence of levels of NADH that promote maximal NADH oxidase activity (16) enables O$_2$ production by this system to exceed the capacity of SOD activity in the arterial homogenate to attenuate the inhibitory effects of this O$_2$ species on sGC activity.

Lactate and pyruvate were employed to modulate cytosolic NAD(H) redox through the effects of these substances on the function of the lactate dehydrogenase reaction (2). We previously observed (22) that lactate potentiated the increase in the detection of O$_2$ by lucigenin caused by inhibition of Cu/Zn SOD as a result of pretreatment of bovine pulmonary arteries with the Cu chelator DETCA. The absence of an effect of 10 mM lactate on relaxation to the NO donors examined is consistent with the anticipated increase in production of both O$_2$ and H$_2$O$_2$ not altering the response to the nitrovasodilators examined. However, the previously reported (5) inhibition of relaxation to NO-releasing agents in bovine pulmonary arteries by the inhibition of SOD with DETCA pretreatment was observed in the present study to be potentiated in the presence of 10 mM lactate. The reversal of the effects of DETCA pretreatment plus lactate on relaxation to the NO donor SNAP by the presence of added SOD (or Tiron) implicates O$_2$ as the key mediator of the observed inhibition. Although exogenous SOD is not likely to enter the intracellular environment of the vessel wall, our previous studies have demonstrated that the treatment of bovine arteries with DETCA permits the majority of O$_2$ that is detected by lucigenin to be scavenged by exogenous SOD (21). In contrast, 10 mM pyruvate, a cellular metabolite that could potentially lower cytosolic NADH levels through the lactate dehydrogenase reaction, did not alter the response to SNAP directly, but it attenuated the inhibitory effects of inactivation of SOD with DETCA pretreatment. In general, the pattern of actions of the various probes employed does not appear to be consistent with a detectable action of the vascular pretreatment with the Cu chelator DETCA influencing the generation of NO by SNAP or with a direct influence of lactate, pyruvate, or the addition of SOD on the response to SNAP. However, these observations are consistent with the hypothesized model shown in Fig. 8, where lactate and pyruvate control the inhibitory effects of inactivation of Cu/Zn SOD on cGMP-associated relaxation to NO donors as a result of their influence on cytosolic NAD(H) redox status and the O$_2$-producing activity of NADH oxidase.

The activity of SOD and redox status of cytosolic NAD(H) are likely to have a major role in the control of vascular sGC activity by NO mechanisms. Observations in the present study are consistent with the activity of SOD in intact bovine pulmonary arterial smooth muscle being sufficient for lowering the endogenous levels of O$_2$ below the concentration range where it significantly inhibits the stimulation of sGC by NO.
Pathophysiologically states that lower the levels of SOD in the vessel wall are likely to exhibit a O₂-mediated impairment of relaxation to NO-dependent vasodilators in a manner similar to the effects of inhibition of SOD with DETCA. In contrast to the effects of inhibition of SOD in the intact muscle, it was observed in the homogenate experiments that increases in NADH caused an impairment of NO stimulation of sGC in the absence of changes in the activity of SOD. In the homogenate preparation, the presence of a NADH-regenerating system and the impairment of mechanisms that control cytosolic NAD(H) redox result in the maintenance of a level of NADH (0.1 mM) that causes maximal production of O₂, based on the previously measured Michaelis constant for NADH of 8–9 µM for the activity of NADH oxidase in the bovine pulmonary artery (16, 30). This may exceed the ability of SOD activity in the homogenate from keeping O₂ levels below the concentration range where it significantly inhibits the stimulation of sGC by NO. Previous studies in the intact bovine pulmonary arterial smooth muscle suggest that mitochondrial function markedly inhibits the maximal expression of lactate-elicited increases in O₂ detection (30). Thus the cellular mechanisms that control the redox status of cytosolic NADH, such as the mitochondrial shuttle systems for removal of cytosolic NADH, are likely to keep cytosolic NAD(H) primarily in its oxidized form even in the presence of lactate, and this may limit the amount of O₂ produced compared with the conditions in the homogenate where NADH levels are kept at 0.1 mM by the NADH-regenerating system. The combination of these factors may prevent lactate-induced increases in O₂ from reacting levels that inhibit relaxation to NO in vessel segments that have not been exposed to environments that lower endogenous SOD activity.

The results of this study provide novel evidence for the important role of cytosolic NAD(H) redox in controlling the effect of vascular O₂ production on NO-elicited relaxation. Furthermore, the data support the previously suggested (5) essential role of Cu/Zn SOD in the expression of NO-elicited cGMP-mediated relaxation. In addition, studies on sGC activity in the homogenate provide evidence consistent with a very important role for the activity of NADH oxidase and SOD in controlling the function of sGC. Alterations in cytosolic NAD(H) redox in metabolic states that increase lactate [e.g., ischemia (19) and exercise (1)] could potentially have an important role in the attenuation of NO-mediated vascular regulation under pathophysiological conditions where the levels of SOD do not adequately prevent the interaction of NO with O₂.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-31069 and HL-43023 and American Heart Association, New York State Affiliate, Grant 970118.


Received 15 September 1998; accepted in final form 12 January 1999.

REFERENCES


