Nitric oxide-dependent pulmonary vasodilation in polycythemic rats

BENJIMEN R. WALKER, THOMAS C. RESTA, AND LEIF D. NELIN
Vascular Physiology Group, Departments of Cell Biology and Physiology and Pediatrics,
University of New Mexico Health Sciences Center, Albuquerque, New Mexico 87131
Received 3 September 1999; accepted in final form 19 June 2000

Polycythemia causes increased vascular production of nitric oxide (NO), most likely secondary to an effect of elevated vascular shear stress to enhance expression of endothelial nitric oxide synthase (eNOS). Because both polycythemia and increased eNOS expression are associated with chronic hypoxia-induced pulmonary hypertension, experiments were performed to test the hypothesis that increased hematocrit leads to upregulation of pulmonary eNOS and enhanced vascular production of NO independent of hypoxia. Rats were administered human recombinant erythropoietin (rEpo; 48 U/day) or vehicle for 2 wk. At the time of study, hematocrit was significantly greater in the rEpo-treated group than in the vehicle group (65.8 ± 0.7% vs. 45.1 ± 0.5%), although mean pulmonary artery pressure did not differ between treatments. Experiments on isolated, saline-perfused lungs demonstrated similar vasodilatory responses to the endothelium-derived NO-dependent agonist ionomycin in each group. Additional experiments showed that the vasoconstrictor response to the thromboxane mimetic U-46619 was diminished at lower doses in lungs from the rEpo group compared with the vehicle group. However, perfusate nitrite/nitrate concentration after 90 min of perfusion in isolated lungs was not different between groups. Additionally, no difference was detected between groups in lung eNOS levels by Western blot. We conclude that the predicted increase in shear stress associated with polycythemia does not result in altered pulmonary eNOS expression.

METHODS

All experimental procedures used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of the University of New Mexico School of Medicine. Male Sprague-Dawley rats (250–350 g) were used for all experiments.

Experimental Development of Polycythemia

Polycythemia was initiated by subcutaneous implantation of an osmotic minipump containing recombinant human erythropoietin (rEpo; Amgen). Two weeks before study, rats were anesthetized with a mixture of ketamine (90 mg/kg im).
and acepromazine (0.9 mg/kg im) and osmotic minipumps (Alzet) containing either rEpo or its saline vehicle were implanted under pentobarbital anesthesia. Topical and systemic antibiotics were administered at the conclusion of surgery. A concentration of rEpo was chosen to deliver 48 U/day via the minipump. Hematocrit was measured in all animals 2 wk after pump implantation to document rEpo-induced polycythemia.

**Measurement of Pulmonary and Systemic Arterial Pressures in Conscious Rats**

Rats were chronically instrumented with pulmonary artery and femoral artery catheters to examine the possible presence of polycythemia-induced pulmonary and systemic hypertension after rEpo treatment. Two days before study, rats were anesthetized as above, and a polyvinyl catheter was advanced via the right jugular vein into the pulmonary artery as previously described (1). Proper location of the catheter was determined by monitoring the pressure waveform during advancement. In addition, in some animals, a polyethylene catheter was introduced via the left femoral artery and advanced into the abdominal aorta. Both catheters were then routed subcutaneously to a point on the dorsal surface of the neck, exteriorized, and placed in a protective plastic container sutured to the rat’s skin. Antibiotics were administered as described for experimental polycythemia.

At the time of study, rats were placed in a Plexiglas box (25 × 15 × 10 cm) containing fresh bedding and flushed with room air. The catheters were opened and flushed with sterile, heparinized saline, and pulmonary artery and systemic arterial pressures were measured with Spectramed P23 XL transducers. Both mean and pulsatile pressures were monitored with a Gould RS-3600 recorder. Rats were allowed 30–60 min to equilibrate to their surroundings. On achievement of stable pressure measurements, mean pressures were averaged over a 10-min period for animals in each group.

**Isolated, Perfused Lung Experiments**

Animals were anesthetized with pentobarbital sodium (32.5 mg ip), and the lungs were isolated for recirculating perfusion with a physiological saline solution (PSS) as previously described (3). After the trachea was cannulated with a 17-gauge needle stub via an incision in the right ventricle and the pulmonary artery was cannulated with a 13-gauge needle stub via an incision in the right ventricle. The preparation was immediately perfused at 0.8 mL/min by a Masterflex microprocessor pump drive (model 7524–10) with PSS containing (in mM) 129.8 NaCl, 5.4 KCl, 25 NaHCO3, 1.8 CaCl2, and 5.5 glucose with 4% albumin (wt/vol) and 10 μg/ml meclofenamate (all from Sigma). The left ventricle was cannulated with a 4-mm-OD plastic tube, and the heart and lungs were removed en bloc and suspended in a humidified chamber at 38°C. The perfusion rate was gradually increased to 30 mL·min⁻¹·kg body wt⁻¹ and was maintained at this rate for the duration of the experiment. Twenty milliliters of perfusate were washed through the lungs and discarded before recirculation was initiated with forty milliliters of PSS. Experiments were performed with the lungs in zone 3 conditions, achieved by elevating the perfusate reservoir until venous pressure was ~3.5 mmHg. Arterial and venous pressures were monitored continuously with Spectramed P23 XL transducers and recorded on a Gould RS-3400 chart recorder. In addition, data were acquired and stored on a personal computer using AT-CODAS software and hardware (Dataq) for later analysis. After isolation, lungs were allowed to equilibrate for 30 min before initiation of one of the following protocols. Lungs showed no sign of edema formation during the entire course of the study, and there was no noticeable fluid loss from the recirculating reservoir. We (4) previously determined that similarly prepared lungs demonstrate a net fluid flux of ~10 μL·min⁻¹·g tissue⁻¹, which includes evaporative loss from the reservoir. Perfusate gas tension measurements were not taken; however, we (2) previously reported values for this preparation.

**Dose Response to U-46619**

Experiments were performed to determine whether polycythemic rats demonstrated different vasoconstrictor responsiveness to the thromboxane mimetic U-46619 (9,11-dideoxy-9α,11o-methanoepoxyprostaglandin F2α, Cayman Chemical) compared with controls. After equilibration, baseline capillary pressure (Pc) was assessed by a double-occlusion technique described previously (3) to permit calculation of arterial and venous resistances. A cumulative dose-response curve to U-46619 (50, 100, 200, and 400 nM) was then generated in lungs from rEpo- and vehicle-treated rats. Stable pressor responses to each dose of U-46619 were allowed to develop before administration of subsequent doses. Pc was assessed at the plateau of the pressor response to each concentration of U-46619 by double occlusion.

**Vasodilatory Responses to Ionomycin**

Additional experiments were performed to examine the possible effects of rEpo-induced polycythemia on the endothelium-derived NO (EDNO)-dependent dilatory response to ionomycin. Ionomycin was chosen for study because it elicits receptor-independent dilations in this preparation that correlate in magnitude with vascular eNOS protein levels (19). After baseline occlusion measurements, lungs were constricted with U-46619 to elicit an arterial pressor response of ~10 mmHg. U-46619 generates stable arterial and venous constriction, allowing subsequent determination of the segmental profile of ionomycin-induced vasodilatory responses. On attainment of a stable vasoconstrictor response, Pc was assessed by double occlusion, and then 350 nM ionomycin (Calbiochem) was added to the reservoir. Pc was again measured at the peak of the vasodilatory response to ionomycin.

**Assessment of Nitric Oxide Production in Lungs From Control and Polycythemic Rats**

Lungs from both groups were isolated and perfused as before, except that no occlusions were performed and no drugs were added to the perfusate. An additional group of lungs was perfused with PSS containing the NOS inhibitor Nω-nitro-l-arginine (l-NNA; 300 μM). As a positive control, lungs were perfused from rats receiving either lipopolysaccharide (LPS, 20 mg/kg ip; n = 4) or saline vehicle (n = 4) 5 h before isolation. One-milliliter samples of perfusate were taken at 0 and 90 min and frozen for later analysis. NOx (NO, NO2, NO3) was determined in each sample using the Griess reaction as described in NOx Assay. The NOx concentration at 90 min was subtracted from that at 0 min to determine the production over that period of time.
**NOx Assay**

The perfusate samples were assayed spectrophotometrically in duplicate for NOx as previously described (13). NADPH (50 μl, 0.8 mg/ml) and nitrate reductase (10 μl, 5 U/ml) were added to 500 μl of lung perfusate, and the mixture was incubated for 3 h at room temperature; then 300 μl of Griess reagent [1% sulfanilamide, 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride, and 2.5% phosphoric acid] was added and incubated for 10 min at room temperature. Absorbance was read at 546 nm using a blank perfusate solution with added NADPH, nitrate reductase, and the Griess reagent. Absorbance values were compared with a standard curve generated by adding known amounts of NaNO3 to the perfusate solution and assaying as described for the samples.

**Western Blotting for eNOS**

Separate sets of rats from each group were anesthetized with pentobarbital sodium (32.5 mg ip), and their lungs were quickly isolated and snap-frozen in liquid nitrogen. For comparison, lungs from chronically hypoxic rats [4 wk at barometric pressure (Pbaro) = 380 mmHg] and from a parallel normoxic group were also harvested. Whole left lungs were fragmented with a mortar and pestle cooled in liquid nitrogen and then homogenized on ice in 10 mM Tris-HCl buffer (pH 7.4) containing 255 mM sucrose, 2 mM EDTA, 12 μM leupeptin, 1 μM pepstatin A, 0.3 μM aprotinin, and 1 mM phenylmethylsulfonyl fluoride (all from Sigma). Tissue homogenates were centrifuged at 1,500 g at 4°C for 10 min to remove cellular debris. The supernatant was collected and centrifuged at 100,000 g at 4°C for 60 min to isolate the microsomal fraction containing eNOS. Protein concentrations of samples were determined by the Bradford method (Bio-Rad protein assay). Tissue sample proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 7.5% acrylamide. The separated proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad) and blocked overnight at 4°C with 5% nonfat milk, 3% bovine serum albumin (Sigma), and 0.1% Tween-20 (Bio-Rad) in a Tris-buffered saline solution (TBS) containing 10 mM Tris-HCl and 50 mM NaCl (pH 7.5). Blots were incubated for 4 h at room temperature with a mouse monoclonal antibody raised against human eNOS (1:2,500) (Transduction Laboratories) in TBS. Immunochemical labeling was achieved by incubation for 1 h at room temperature with a horseradish peroxidase-conjugated goat anti-mouse IgG (1:5,000) (Bio-Rad) in TBS followed by chemiluminescence labeling (Amersham ECL). eNOS protein bands were detected by exposure to chemiluminescence-sensitive film and quantitated by densitometric analysis (Sigma Gel, Jandel Scientific). Membranes were stained with Coomassie brilliant blue to confirm equal protein loading between lanes.

**Assessment of Right Ventricular Hypertrophy**

Possible right ventricular hypertrophy was assessed as an index of polycythemia-induced pulmonary hypertension. Briefly, cardiac tissue was isolated from the same rats used for lung harvesting for Western blot. After isolation of the heart, the atria and major vessels were removed from the ventricles, and the right ventricle was dissected from the left ventricle and septum. The degree of right ventricular hypertrophy was assessed by calculating the ratio of right ventricle weight (RV) to total ventricular weight (T).

**Data Analysis and Statistics**

Total pulmonary vascular resistance in isolated, perfused lungs was calculated in duplicate as the difference between arterial pressure and venous pressure divided by flow. Pulmonary arterial resistance was calculated as the difference between arterial pressure and Pp divided by flow. Similarly, pulmonary venous resistance was calculated as the difference between Pp and venous pressure divided by flow. Vasodilatory responses were calculated as a percent reversal of U-46619-induced vasoconstriction for the total pulmonary vasculature as well as for arterial and venous segments.

All data are expressed as means ± SE. Values of n refer to the number of animals in each group. Where appropriate, one-way ANOVA, two-way ANOVA, or Student’s t-test was used to make comparisons. If differences were detected by ANOVA, individual groups were compared using the Student-Newman-Keuls test for all pairwise comparisons. All data expressed as percentages were normalized using the arcsine transformation before analysis with appropriate parametric tests. A level of P ≤ 0.05 was accepted as statistically significant for all comparisons.

**RESULTS**

**Effect of rEpo Administration on Hematocrit and Pulmonary Arterial Pressure**

Hematocrit was significantly elevated in rats administered rEpo compared with vehicle. Figure 1 shows the pooled hematocrit data from animals in all protocols. Hematocrit was assessed before lung isolation or after measurement of vascular pressures in conscious rats. There were no differences between rEpo-treated rats in the various protocols. Similarly, hematocrit did not differ between vehicle-treated animals assigned to different protocols. The degree of polycythemia attained with rEpo treatment is very similar to that observed in chronically hypoxic rats in our laboratory (18, 19). Despite the significantly elevated hematocrit, mean pulmonary arterial pressure did not differ significantly between rEpo (n = 7)- and vehicle (n = 5)-treated rats (Fig. 2A). Consistent with this finding, right ventricular hypertrophy was not observed in the rEpo-treated rats compared with controls. The calculated ratio RVT was 0.216 ± 0.005 (n = 6) in vehicle-treated rats and 0.212 ± 0.003 (n = 6) in rats receiving rEpo. Similarly, neither right ventricular mass/body weight nor
(left ventricular + septum mass)/body weight was different between groups. In contrast, systemic arterial pressure (Fig. 2B) was significantly elevated in rEpo-treated rats \((n = 4)\) compared with controls \((n = 4)\), as previously reported by others \((11, 12, 26, 29)\).

**Isolated Lung Experiments**

**Baseline perfusion pressure and segmental vascular resistances.** Initial arterial pressure averaged \(6.6 \pm 0.1\) mmHg and did not differ between groups. Venous pressure was set as described in METHODS. Table 1 shows total, arterial, and venous baseline resistances in lungs from each group of animals in which vasoreactivity measurements were made. No differences in either total or segmental resistance were noted between groups, suggesting that rEpo-induced polycythemia is not associated with vascular remodeling sufficient to alter basal resistance.

**Dose response to U-46619.** Cumulative dose-response data illustrating responsiveness to U-46619 are shown in Fig. 3. Lungs from rEpo-treated rats demonstrated lesser total vasoconstrictor responses to U-46619 than lungs from vehicle-treated animals at the 50 and 100 nM doses as well as a tendency for a similar reduction at higher doses of the agonist. This difference in the total response was attributable to reduced sensitivity in the venous segment, in which statistical differences were noted at the 50 and 200 nM doses. Arterial vasoconstrictor responses to U-46619 did not differ between groups.

**Vasodilatory responses to ionomycin.** Levels of pre-constriction before ionomycin administration did not differ between groups, although there was a tendency for the requirement of slightly greater concentrations of U-46619 to achieve constriction in the rEpo group (Table 2). Figure 4 presents the vasodilatory responses to 350 nM ionomycin in U-46619-constricted lungs. No differences in the total vasodilatory response to ionomycin were observed between lungs from rEpo- and vehicle-treated animals. Similarly, arterial and venous vasodilatory responses to ionomycin were not different between groups.

**Table 1. Baseline segmental vascular resistances in isolated, perfused lungs**

<table>
<thead>
<tr>
<th></th>
<th>Total (mmHg)</th>
<th>Arterial (mmHg)</th>
<th>Venous (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>17</td>
<td>0.072 ± 0.005</td>
<td>0.030 ± 0.004</td>
</tr>
<tr>
<td>rEpo</td>
<td>15</td>
<td>0.064 ± 0.006</td>
<td>0.021 ± 0.003</td>
</tr>
</tbody>
</table>

Values (in mmHg·ml⁻¹·min⁻¹·kg body wt) are means ± SE; \(n\), number of rats. rEpo, recombinant human erythropoietin. There were no differences between the groups.
Table 2. U-46619 doses and levels of segmental preconstriction for ionomycin vasodilatory experiments in isolated, perfused lungs

<table>
<thead>
<tr>
<th>U-46619 Dose, nM</th>
<th>Δ Total R</th>
<th>Δ Arterial R</th>
<th>Δ Venous R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle 149.1 ± 8.4</td>
<td>0.310 ± 0.011</td>
<td>0.198 ± 0.013</td>
<td>0.112 ± 0.012</td>
</tr>
<tr>
<td>rEpo 184.2 ± 23.2</td>
<td>0.326 ± 0.014</td>
<td>0.217 ± 0.011</td>
<td>0.113 ± 0.013</td>
</tr>
</tbody>
</table>

Values are means ± SE. Changes (Δ) in resistance (R) are in mmHg·ml⁻¹·min⁻¹·kg body weight. There were no differences between the groups.

Perfusate NO₃⁻ from lungs from vehicle- and rEpo-treated rats. NO₃⁻ concentration after 90 min of perfusion did not differ between rEpo- and vehicle-treated groups (Fig. 5A). In contrast, NO₃⁻ concentration after 90 min of perfusion in control lungs treated with the NOS inhibitor L-NNA was significantly less than in lungs from rEpo- or vehicle-treated rats without L-NNA (Fig. 5A). In addition, lungs from rats treated with LPS demonstrated markedly increased NO₃⁻ concentrations compared with a parallel vehicle-treated group (Fig. 5B), illustrating the ability of the assay to detect differences in pulmonary NO production to known inhibitory and stimulatory influences.

Western Blotting for eNOS

Immunoreactive eNOS was detected in all lung samples; however, no significant difference in protein levels was detected between vehicle and rEpo groups using densitometric analysis of the blots (Fig. 6A). In contrast, lungs from chronically hypoxic rats demonstrated significantly greater eNOS levels than a parallel normoxic control group (Fig. 6B).

DISCUSSION

The present study examined the putative role of polycythemia on NO-dependent vasodilator responses in the pulmonary circulation. The major findings of this study were as follows. 1) Administration of rEpo to normoxic rats resulted in polycythemia closely mimicking that induced by chronic hypoxia previously shown to be associated with elevated eNOS gene expression. 2) Although hematocrit was significantly elevated, baseline pulmonary artery pressure was not increased in rEpo-treated animals. 3) Vasoconstrictor responses to U-46619 were diminished in lungs from rEpo-treated rats; however, vasodilator responses to the EDNO-dependent dilator ionomycin were unaffected. 4) Basal pulmonary NO production was unaltered by polycythemia. 5) No difference in pulmonary eNOS levels was detected between vehicle- and rEpo-treated rats. These data suggest that polycythemia alone is an insufficient stimulus to increase eNOS expression or activity in the pulmonary vasculature.

A number of studies have shown that unidirectional shear stress in the physiological range (1.2–25 dyn/cm²) is a regulator of several genes including expression of the eNOS gene (17, 25, 30). Because laminar shear stress is proportional to perfusate viscosity, increased blood viscosity secondary to polycythemia has been postulated to enhance eNOS gene expression. Consistent with this hypothesis, Wilcox and co-workers (29) reported that rats made polycythemic by rEpo administration exhibited greater pressor responses to NO inhibition than control animals, suggesting enhanced basal NO production in the polycythemic group. Similar results have been reported in spontaneously hypertensive rats administered rEpo (12). Recent studies have confirmed that NO production is elevated systemically by rEpo-induced polycythemia. Two separate studies documented increased urinary excretion of NO metabolites in polycythemic compared with control animals (11, 23). In addition, Tsukahara et al. (23) observed that a specific inhibitor of inducible NOS (iNOS) activity did not affect this elevated NO₃⁻ excretion, whereas nonspecific NOS inhibition was effective.
These latter data suggest that the elevated systemic NO production evident in polycythemia is most likely caused by enhanced eNOS activity. In addition, it appears that this elevated eNOS activity is caused by increased shear stress and not by a direct action of rEpo, because rEpo administration to cultured endothelial cells is without effect on eNOS levels (10) or may even cause a reduction in expression at high concentrations of the hormone (28). Thus systemic production of NO appears to be stimulated by polycythemia; however, the relative contributions of specific sites such as the lung toward this production are not clear.

In the present study, we observed no difference in pulmonary vasodilatory responses to the EDNO-dependent agent ionomycin or in basal production of NO in lungs from control and polycythemic rats. The use of U-46619 to constrict the lungs allowed subsequent assessment of segmental responses to ionomycin. Furthermore, vasodilatory responses to ionomycin are the result of direct stimulation of eNOS by calcium entry and are thus not complicated by possible alterations in ligand-dependent signal transduction. Similar to our results, Migliori et al. (11) failed to detect enhanced responses to endothelium-dependent dilators in the renal circulation after rEpo administration, although urinary NOx excretion was greater than in controls. Thus there may be regional heterogeneity of the sites of enhanced eNOS expression during polycythemia. Despite the failure of rEpo treatment to increase basal NO production in the lung, we did observe slightly reduced vasoconstrictor responses to U-46619 in the polycythemic rats. This reduced reactivity was restricted to the venous circulation and is not likely related to altered NO production. The mechanism for this response is unclear.

Expression of eNOS within both the pulmonary circulation and hematocrit are increased during chronic hypoxia (8, 18–20, 24). The mechanism(s) underlying the upregulation of eNOS in the lung in response to this stimulus is controversial. Data from our laboratory support the involvement of altered mechanical forces such as shear stress in this response. For example, eNOS protein levels are selectively increased in the pulmonary arterial circulation, where shear forces are the highest because of the combination of polycythemia and vascular remodeling (19), although the quantitative effect of these factors in vivo is impossible to determine. Furthermore, rats initially exposed to chronic hypoxia but subsequently returned to normoxia for up to 2 wk demonstrate sustained polycythemia, pulmonary vascular remodeling, and elevated eNOS gene expression compared with controls, suggesting that mechanical forces rather than hypoxia per se may be responsible for this response (18). In contrast, Le Cras and co-workers (8) recently found that, even after the left pulmonary artery was banded to minimize shear forces, eNOS expression was still stimulated by hypoxic exposure in the banded lung. These latter data support a role of hypoxia independent of increased shear stress or pulmonary hypertension in the regulation of eNOS expression. In the present study, we examined the effect of polycythemia independent of hypoxia on NO-dependent dilation and basal NO production in the lung. Although we were able to detect stimulated pulmonary NO production after systemic LPS treatment in isolated lungs, we observed no differences between vehicle- and rEpo-treated groups. Whereas it is possible that our methodology lacks the sensitivity to detect small changes in NO production in the isolated lung, our findings suggest that polycythemia alone is an insufficient stimulus to significantly alter NO production in the normoxic pulmonary circulation. Furthermore, we failed to demonstrate a difference in immunoreactive eNOS in lung tissue from polycythemic versus control rats. In contrast, we did observe greater eNOS levels in lungs from chronically hypoxic rats compared with a parallel normoxic group, which is consistent with our earlier published results. Although these results could be interpreted to support a direct causative role of oxygenation on eNOS regula-
tion during chronic hypoxia, it is also possible that reduced pulmonary arterial luminal diameters associated with remodeling could exacerbate the effects of polycythemia beyond those observed in the normoxic lung.

Polycythemia is associated with increased blood viscosity and thus would be predicted to result in hypertension. Indeed, administration of rEpo to rats has been shown to elicit systemic hypertension, although factors other than increased blood viscosity may contribute to the response (26, 27). Consistent with these earlier findings, we observed a significant elevation in mean arterial pressure in rats treated with rEpo compared with controls. Previous studies have suggested that enhanced NO production may protect against the development of more severe systemic hypertension in rEpo-treated rats (29). In contrast, we did not observe any significant effect of rEpo-induced polycythemia on pulmonary artery pressure in conscious rats or any effect on right ventricular mass. These results are similar to those of Petit and co-workers (14), who found no difference in the amount of right ventricular hypertrophy or systolic right ventricular pressure in chronically hypoxic rats administered rEpo versus hypoxic controls. In a subsequent report (15), these investigators documented lower baseline vascular resistance in lungs isolated from rEpo-treated hypoxic rats compared with hypoxic controls, which was attributable to reduced vascular remodeling in the polycythemic rats. In contrast, we did not observe differences in baseline vascular resistance in lungs isolated from rEpo- versus vehicle-treated normoxic rats, suggesting that other factors such as increased vascular recruitment may be responsible for the comparable in vivo pressures observed. Such factors also make the effect of polycythemia on local shear stress within the lung difficult to predict; however, it is possible that shear-induced release of NO could potentially offer a protective effect against the development of pulmonary hypertension despite the lack of apparent upregulation of eNOS expression. Furthermore, other factors could contribute to lack of pulmonary hypertension in this setting. For example, it is possible that the increased concentration of red blood cells could contribute additional vasodilatory influences via the release of ATP or other metabolites that could alter vascular tone (5, 6, 22). Additionally, it is possible that the release of other vasodilatory factors such as prostacyclin or endothelium-derived hyperpolarizing factors could account for the failure of polycythemia to elicit pulmonary hypertension.

In summary, the present study detected no evidence for enhanced pulmonary vascular NO production or eNOS expression associated with polycythemia induced by rEpo. Thus the enhanced eNOS expression observed in the pulmonary circulation after chronic hypoxia cannot likely be attributed to alterations in hematocrit alone.

The authors thank Minerva Murphy, Heather Nash, and Jay Naik for their assistance.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-52184 (B. R. Walker) and HL-09660 (T. C. Resta) and by a Scientist Development Grant from the American Heart Association (T. C. Resta). T. C. Resta is a Parker B. Francis Fellow in Pulmonary Research.

REFERENCES


