Nitric oxide regulation of microvascular oxygen exchange during hypoxia and hyperoxia

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HYPEROXIA AND HYPOXIA MODULATE oxygen delivery to the tissue by a variety of central and local mechanisms the end point of which is to reduce vascular resistance in hypoxia, and conversely to elevate it in hyperoxia, to maintain oxygen delivery to the tissue at a constant level. The classical explanation for this process is based on the formation of biochemical vasodilator signals generated by the parenchyma in response to inadequate oxygen supply to the tissue. These signals are transmitted from parenchymal cells to the resistance vessels, controlling vessel tone, allowing blood flow to vary to meet metabolic needs (25). A second mechanism involves prostanoids the release of which from endothelial cells is enhanced in response to lowering tissue PO2 levels (2), an effect also present at high PO2 levels. It has been shown in studies on isolated arterioles from the rat cremaster muscle that elevating oxygen above normal tissue levels causes vasoconstriction because of release inhibition of prostanoids from the endothelium (22).

Nitric oxide (NO) is a pervasive vasoactive material. However, the impact of elevated or decreased oxygen tension on NO synthesis has not been clearly established by studies with cell cultures and isolated enzymes (23). Vasodilatation induced by hypoxia is related to the release of adenosine by the endothelium when local PO2 decreases (21, 30). An additional effect due to hypoxia is the inhibition of endothelial respiration in the presence of NO (12), which renders endothelial cell oxygen consumption dependent on oxygen concentration (6). A relationship between endothelial cell, smooth muscle oxygen consumption, and local intravascular PO2 was found by comparing microvessel wall oxygen concentration gradients and local blood PO2 in vivo (28, 33). Microvascular wall oxygen gradients are directly related to vessel wall oxygen consumption, which previous studies have shown increases in direct relation to local blood PO2 (34) and the level of microvascular tone (15, 29).

The present study analyzes the microvascular effects of hyperoxia and hypoxia combined with conditions where NO availability is maximally reduced to determine how oxygen delivery and consumption are affected by NO. Our aim was to test the hypothesis that increased NO availability lowers vascular constriction due to oxygen oversupply by decreasing the amount of oxygen consumed by the arteriolar vessel wall. We propose that lowering oxygen consumption in the vessel wall reduces energy expenditure, thus limiting the capacity to produce the mechanical work necessary for maintaining sustained constriction against blood pressure. An alternate view is that reducing NO availability increases oxygen consumption by the tissue (27).

The NO scavenger used in this study was the nitronyl nitroxide compound oxidized and reduced form of 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO), which has been reported by Akaike et al. (1) to react stoichiometrically with NO to generate NO2 and a 2-carboxy-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl (PTI) derivative. Carboxy-PTIO is water soluble and has been shown to have very strong NO-scavenging properties based on a radical-radical reaction with NO (1).
planted in the carotid artery and jugular vein (4, 32). The experiment was performed after at least 24 h but within 48 h of catheter implantation.

Inclusion criteria. Animals were suitable for the experiments if 1) systemic parameters were within normal range, namely, heart rate (HR) >340 beats/min, mean arterial blood pressure (MAP) >80 mmHg, systemic hematocrit (Hct) >45%, and arterial PO2 >50 Torr; and 2) microscopic examination of the tissue in the chamber observed under a ×65 magnification did not reveal signs of edema or bleeding. Hamsters are a fossorial species with a lower arterial PO2 than other rodents because of their adaptation to the subterranean environment. However, microvascular PO2 distribution in the skin back window model is the same as in other rodents such as mice (3).

Materials. All chemicals were of an analytical grade. Carboxy-PTIO was purchased from Cayman Chemical. A stock solution was made using a shaping slit in a rectangular format, which is placed a distance from centrifuged arterial blood samples taken in heparinized capillary tubes. Hemoglobin content was determined spectrophotometrically from a single drop of blood (B-Hemoglobin, Hemocue, Stockholm, Sweden).

Functional capillary density. Functional capillaries, defined as those capillary segments that have red blood cell (RBC) transit of at least a single RBC in a 30-s period, in 10 successive microscopic fields were assessed, totaling a region of 0.46 mm². Each field had between two and five capillary segments with RBC flow. Functional capillary density (FCD cm⁻¹), i.e., total length of RBC perfused capillaries divided by the area of the microscopic field of view, was evaluated by measuring and adding the length of capillaries that had RBC transit in the field of view. The relative change in FCD from baseline levels, after each intervention, is indicative of the extent of capillary perfusion (4, 32).

Microhemodynamics. Arteriolar and venular blood flow velocities were measured online by using the photodiode cross-correlation method (16) (Photo Diode/Velocity Tracker model 102B, Vista Electronics, Ramona, CA). The measured centerline velocity (V) was corrected according to vessel size to obtain the mean RBC velocity (20). A video image-shearing method was used to measure vessel diameter (D) (17). Blood flow (Q) was calculated from the measured values as Q = V·πD²/4. Changes in arteriolar and venular diameter from baseline were used as indicators of a change in vascular tone.

Hemoglobin-oxygen equilibrium curves. Oxygen saturation of freshly collected hamster blood was investigated by deoxygenation of oxygen-equilibrated oxyhemoglobin in a Hemox buffer (7.28, 7.35, and 7.49) at 37.6°C, using a Hemox Analyzer (TCS Scientific, New Hope, PA). Changes in pH were set adding Tris and bis-Tris buffers to the Hemox buffer. Tris and bis-Tris buffers were prepared by fully titrating the reagents with HCl before adjusting the pH of the solutions. In this way, the concentration of Cl⁻ ions was equal to that of the buffer at all pH values.

Microvascular PO2 distribution. High-resolution microvascular PO2 measurements were made using phosphorescence-quenching microscopy (31). This method for measuring oxygen levels is based on the oxygen-dependent quenching of phosphorescence emitted by albumin-bound metalloporphyrin complex after pulsed light excitation. The phosphorescence decay curves were converted to oxygen tensions using an fluorescence decay-curve fitter (model 802, Vista Electronics, Logan, UT). The dye was allowed to circulate for 10 min before Po2 measurements (18, 31, 34).

In our system, intravascular measurements are made by placing an optical rectangular window (5 × 15 μm) within the vessel of interest, with the longest side of the rectangular slit positioned parallel to the vessel wall. Tissue PO2 is measured in regions void of large vessels within intercapillary spaces (10 × 10 μm) (33). In the present configuration of the oxygen-measuring system, tissue PO2 values are obtained with a repeatability of 1–3 Torr capturing the emission from an area related to the tissue of ∼75–100 μm² (31). Measurements in regions with large tissue gradients in the vicinity of arterioles are made using a shaping slit in a rectangular format, which is placed a long the outside of the vessel wall, and by varying the length the acceptable signal-to-noise ratio was obtained (18). Perivascular PO2 measurements are made by placing the centerline of the measuring slit at a distance that is one-tenth of the inner vessel diameter that stops at the blood tissue interface.

Tissue oxygen delivery and extraction. Calculations of oxygen delivery and extraction (3–5, 7), are made using Eqs. 1 and 2:

\[ O_2\text{ delivery} = [(\text{RBC}_{\text{Hb}} \cdot \gamma \cdot S_{\text{A,VO2}}) + (1 - \text{Hct}) \cdot \alpha \cdot \text{PO}_{2}\text{AV}] \cdot Q \] (1)

\[ O_2\text{ extraction} = [(\text{RBC}_{\text{Hb}} \cdot \gamma \cdot S_{\text{A,VH}}) + (1 - \text{Hct}) \cdot \alpha \cdot \text{PO}_{2}\text{AV}] \cdot Q \] (2)

where RBC, is the hemoglobin in RBCs (g hemoglobin/dl blood), γ is the oxygen-carrying capacity of hemoglobin (1.34 ml O2/g hemoglobin), Sα is the arteriolar oxygen saturation, (1 - Hct) is the blood fraction of plasma, α is the solubility of oxygen in plasma (3.14 × 10⁻³ ml O2/dl Torr), PaO₂ is the arterial PO2, Q is the microvascular flow relative to baseline, and the subscript A-V indicates the difference between arterioles and venules.

Experimental setup. The unanesthetized animal was placed in a restraining tube with a longitudinal slit from which the window chamber protruded, and then it was fixed to the microscopic stage of a transillumination intravital microscope (BX51WI, Olympus, New Hyde Park, NY). The animals were given 20 min to adjust to the change in the gas environment before measurements. The tissue image was projected onto a charge-coupled device camera (COHU 4815) connected to a videocassette recorder and viewed on a monitor. Measurements were carried out using a ×40 (LUMPFL-WIR, numerical aperture 0.8, Olympus) water-immersion objective. The same sites of study were followed throughout the experiment so that comparisons could be made directly to baseline levels. The animals were randomly started with low or high oxygen followed by the opposite (high or low) to reduce the bias based on the order of gas exposure. After 10 min of exposure, systemic parameters, FCD, vessel diameter, velocity, and intravascular oxygen tension were measured.

Data analysis. Results are presented as means (SD) unless otherwise noted. Data within each group were analyzed using analysis of variance for repeated measurements (ANOVA, Friedman test). When
appropriate, post hoc analyses were performed with the Dunn’s multiple comparison test. All data are presented as absolute values and ratios relative to baseline values. A ratio of 1.0 would signify no change from baseline, whereas lower and higher ratios are indicative of changes proportionally lower and higher than baseline (i.e., 1.5 would mean a 50% increase from the baseline level). All measurements were compared with baseline levels obtained before the experimental procedure. The same vessels and functional capillary fields were followed so that direct comparisons to their baseline levels could be performed, allowing for more robust statistics for small sample populations. All statistics were calculated using GraphPad Prism 4.01 (GraphPad Software, San Diego, CA). Changes were considered statistically significant if $P < 0.05$.

RESULTS

Animal distribution and dose response. Four hamsters [63.9 g body wt (SD 5.2)] were used to study the dose response to a continuous infusion of carboxy-PTIO. A maximal increase in MAP [154 mmHg (SD 14)] was obtained during an infusion of 1.4 mg·kg$^{-1}$·min$^{-1}$ (Fig. 1). From this finding, the dosage of 1.0 mg·kg$^{-1}$·min$^{-1}$ [MAP = 152 mmHg (SD 12) during normoxia] was used to study the effect of NO scavenging on hypoxia and hyperoxia. The small difference in MAP did not justifiy the use of 1.4 mg·kg$^{-1}$·min$^{-1}$ compared with 1.0 mg·kg$^{-1}$·min$^{-1}$. Twelve animals were entered into the hyperoxia/hypoxia study; 6 received a continuous infusion of the NO scavenger and 6 the continuous infusion of the vehicle.

Systemic and blood-gas parameters. Alteration in the inspired gas (FIO$_2$ = 0.1 or FIO$_2$ = 1.0) did not significantly modify MAP and HR in the animal that received vehicle. In the group of animals continuously infused with carboxy-PTIO, MAP increased significantly during normoxia [148.7 mmHg (SD 7.5)], increased further during hyperoxia [167.1 mmHg (SD 8.7)], and significantly decreased from normoxia when oxygen was decreased (FIO$_2$ = 0.1; 128.0 ± 7.4 mmHg).

The inhalation of 10% and 100% oxygen caused 1) significant changes in arteriolar PO$_2$, 2) modified arterial PCO$_2$, and 3) significantly changed arterial pH. These changes were expected and may be the consequence of hypoventilation and hyperventilation, a normal response to hypoxia and hyperoxia. Table 1 summarizes the changes in systemic parameters in response to hypoxia and hyperoxia during infusion of carboxy-PTIO and the vehicle.

Microhemodynamics. The microvascular diameter responses to FIO$_2$ = 0.1 or 1.0 with and without the NO scavenger are shown in Fig. 2. The continuous infusion of the NO scavenger during normoxia induced significant arteriolar vasoconstriction [0.83 (SD 0.09), ratio relative to baseline, n = 24], whereas venular changes were not significant [0.92 (0.10), n = 18]. A decrease in FIO$_2$ resulted in a statistically significant increase in arterioles diameter [1.12 (SD 0.08)] for the vehicle. NO scavenging and the decrease in FIO$_2$ maintained significant arteriolar vasoconstriction [0.92 (SD 0.08)]. In the hypoxic conditions both with and without the NO scavenger, venular diameter changes were not significantly different from normoxia. An increase in FIO$_2$, resulted in a statistically significant diameter decrease in arterioles [0.94 (0.06)] for the vehicle. The NO scavenger and the increase in FIO$_2$ significantly increased arteriolar vasoconstriction [0.79 (0.08)]. During hyperoxia with the NO scavenger and without it, venular diameter changes were not significantly different from normoxia.

Diameter and RBC velocity data were used to compute microvascular blood flow in each vessel studied. Figure 2 shows the change of blood flow in arterioles and venules after FIO$_2$ = 0.1 and FIO$_2$ = 1.0, with and without the NO scavenger. Inhalation of high oxygen levels tended to increase RBC flow velocity, but calculated blood flow was lower in all situations compared with normoxia without the NO scavenger. Statistically significant arteriolar and venular flow reductions were only present during hypoxia without the NO scavenger. How-

Table 1. Systemic parameter during hypoxia and hyperoxia with and without no scavenger

<table>
<thead>
<tr>
<th>FIO$_2$</th>
<th>Vehicle</th>
<th>NO Scavenging</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.21</td>
<td>0.10</td>
</tr>
<tr>
<td>$n$</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>103 (5)</td>
<td>105 (7)†</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>418 (23)</td>
<td>468 (41)</td>
</tr>
<tr>
<td>Pao$_2$, Torr</td>
<td>60.4 (7.2)</td>
<td>27.4 (3.2)*†</td>
</tr>
<tr>
<td>Paco$_2$, Torr</td>
<td>53.7 (6.4)</td>
<td>33.7 (4.8)</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.36 (0.02)</td>
<td>7.47 (0.03)†</td>
</tr>
</tbody>
</table>

Values are means (SD); n, no. of animals. Baseline included all the animals in the study. NO, nitric oxide; FIO$_2$, inspired oxygen fraction; MAP, mean arterial blood pressure; Pao$_2$, arterial PO$_2$; Paco$_2$, arterial PCO$_2$. *P < 0.05 vs. normoxia (vehicles). †P < 0.05 vs. normoxia (no scavenger).
ever, venular flow was also statistically significantly decreased during hypoxia with the NO scavenger.

**FCD.** Continuous infusion of the NO scavenger during normoxia statistically significantly reduced FCD to 0.78 (SD 0.09) of baseline. Decrease in \( \text{FiO}_2 \) resulted in a small decrease in FCD [0.94 (SD 0.08)] for the vehicle. The NO scavenger and the decrease in \( \text{FiO}_2 \) further reduced FCD [0.71 (SD 0.09)], which was statistically significantly lower than normoxia and hyperoxia without the NO scavenger.

**Microvascular oxygen distribution.** Figure 3 shows the distribution of \( \text{PO}_2 \) in the microvascular network (arterioles and venules) and in the interstitial space (tissue) for hyperoxic, hypoxic, and normal conditions, with and without the NO scavenger.

**Wall oxygen gradients.** Arteriolar wall gradients were determined from the difference between intravascular and perivas-
cular Po2 measurements measured across the vessel wall. This parameter has been shown to be directly related to the rate of oxygen consumption of the vessel wall (18, 33). The vessel wall gradient in arterioles in normoxia conditions was 16 mmHg (SD 4) [n = 10; diameter 56 μm (SD 8)]. In normoxia during continuous infusion of the NO scavenger, the vessel wall gradient in arterioles was significantly increased to 23 mmHg (SD 3) [n = 12; diameter 54 μm (SD 9)]. Hypoxia decreased the wall oxygen gradient with the NO scavenger to 11 mmHg (SD 3) [n = 8; diameter 57 μm (SD 8)] and without the scavenger to 9 mmHg (SD 3) [n = 8; diameter 55 μm (SD 7)]. Hypoxia increased the wall oxygen gradient to 26 mmHg (SD 4) [n = 10; diameter 58 μm (SD 8)] with the NO scavenger and the vehicle to 22 mmHg (SD 5) [n = 8; diameter 54 μm (SD 5)] (Fig. 4A).

**Oxygen delivery and extraction.** Figure 4B shows the result of the analysis for extraction of oxygen carried out at the microcirculatory level. Calculations of oxygen delivery and extraction [means (SD)] showed that during normoxia without the NO scavenger, oxygen delivery was 7.7 ml O2/dl blood (SD 2.1) and oxygen extraction was 3.1 ml O2/dl blood (SD 0.8), with a extraction ratio of 40%. Continuous infusion of the NO scavenger during normoxia reduced oxygen delivery to 6.3 ml O2/dl blood (SD 1.6) and increased oxygen extraction to 5.6 O2/dl blood (SD 1.4), increasing the extraction ratio to 88%. During hypoxia, the animal that received the vehicle showed a reduction in oxygen delivery to 1.7 ml O2/dl blood (SD 0.6), a reduction in oxygen extraction to 1.6 ml O2/dl blood (SD 0.6), and an increase in the extraction ratio to 93%. The infusion of the NO scavenger during hypoxia decreased oxygen delivery to 2.0 ml O2/dl blood (SD 0.5), decreased oxygen extraction to 1.9 ml O2/dl blood (SD 0.5), and increased the extraction ratio to 95%. Hyperoxia without the NO scavenger, increased oxygen delivery to 8.8 ml O2/dl blood (SD 2.1) and oxygen extraction to 3.5 ml O2/dl blood (SD 0.9), with no change in the extraction ratio of 40% [similar findings were reported previously Tsai et al. (32)]. Hyperoxia during continuous infusion of the NO scavenger increased oxygen delivery to 8.0 ml O2/dl blood (SD 1.8), oxygen extraction to 5.9 ml O2/dl blood (SD 1.3), and the extraction ratio to 74%.

**DISCUSSION**

The principal finding of this study is that a dose of 1.0 mg·kg\(^{-1}\)·min\(^{-1}\) carboxy-PTIO in our hamster chamber window model significantly elevated MAP, increased FIO\(_2\) to 1.0, and increased MAP even higher. FIO\(_2\) 0.1 and NO scavenging elevated MAP relative to the vehicle, but this value was significantly lower than that attained with NO scavenging and normoxia (Table 1). There was no effect on MAP in hyperoxia and hypoxia in the absence of NO scavenging (vehicle). These effects occurred in parallel with changes in the microcirculation where oxygen extraction (i.e., the release of oxygen from blood to the tissue) was significantly increased when the NO was removed with both normoxia and hyperoxia. Furthermore, the vessel wall oxygen gradient, a measurement of the rate of oxygen consumption by the vessel wall, was increased in hyperoxia (vs. normoxia) and further increased by the combination of hyperoxia and NO scavenging. Vessel wall oxygen consumption was also increased by normoxia and NO scavenging, but it was significantly reduced by hypoxia. The calculations of oxygen delivery and uptake should be considered as representative of the trend in these data in view of the number of different measurements that must be used in the calculations. However, these trends are consistent with previously reported results and have further confirmed the findings of Shibata et al. (28, 29), who measured oxygen gradients in the microcirculation of skeletal muscle.

The changes in MAP show how hyperoxia elicits a vasoconstrictor reserve beyond the NO-dependent vascular tone. The experiments of Messina et al. (22) suggest that this effect may be related to the modulation of production of prostacyclin, a mediator not measured in these experiments because of the limited amount of blood available for sampling. The changes in arteriolar diameter, in addition to their reversal with hypoxia,
support the contention that the effects are primarily due to vasoactivity.

The results obtained with 10% oxygen inspiration are anomalous when compared with the findings of Marshall (21), regarding the lowering of MAP consequent to the elicited vasodilatation. This finding suggested that this level of hypoxia in our model is not sufficient to cause the hypoxic responses found by other investigators in rats. This may be due to the adaptation of this species to a fossorial environment and because our experimentation is carried out in unanesthetized animals. When our Hamster model is subjected to inspiring a 5% oxygen (normobaric), MAP decreases and vessel diameter increases, following observations of Marshall. Observation at 5% oxygen was not studied further because the obvious anoxic conditions of the animals affect the physiological responses.

The difference in PO2 across the arteriolar wall, also referred to as the vessel wall oxygen gradient, is a measurement of the oxygen consumption by the constituents of the wall (33). This gradient increases as a function of the effect of vasoconstrictors, such as arginine vasopressin (14) and the administration of N^G-nitro-L-arginine methyl ester (29) and is lowered by vasodilators, such as verapamil (15). The present study shows that the arteriolar wall PO2 gradient is also modulated by the level of blood PO2. The infusion of an NO scavenger increases the wall gradient, and therefore, the wall oxygen consumption, lowering the amount of oxygen available to the tissue, evidenced by the lowering of tissue PO2.

Elevating oxygen tension above ambient level increases NO production by pulmonary endothelial cells in intact lungs (9, 26). Hypoxic condition increases synthesis of NO and release of NO in cells from skeletal muscle causing a redistribution of blood flow (9–11), which should lead to a more homogeneous distribution of oxygen within the muscle. NO and oxygen availability interact because NO is a modulator of tissue oxygen consumption as shown by the studies of Shen et al. (27) and King et al. (19) and corroborated by our findings. Thus increased NO availability increases oxygen delivery in tissue by reducing oxygen demand, and vice versa. Hyperoxia causes vasoconstriction as evidenced by the decrease in FCD, but this phenomenon is compensated for by the decrease in cardiac output shown by the decrease of HR, with the result that in conditions of normal endothelial function and NO availability there is no change in MAP. In a previous study, our laboratory showed that temporary hyperoxic ventilation causes vasoconstriction, reduction in cardiac output, microcirculatory blood flow, and FCD in the hamster window chamber model (32).

FCD was greatest in normoxia conditions, reduced by both hypoxia and hyperoxia, and was further reduced by NO scavenging in all conditions. The reduction of FCD in hyperoxia with and without NO scavenging, as well as hypoxia with NO scavenging, may be due to the reduction of capillary pressure resulting from arteriolar vasoconstriction (4). FCD did not change in hypoxia because of vasodilatation, allowing a more direct transmission of MAP to the capillaries. During hyperoxia tissue PO2 increased, whereas FCD decreased, showing that tissue PO2 is not a direct function of FCD (capillary perfusion) and that it is in part regulated by arteriolar and venular PO2. A direct relationship between tissue and venular PO2 is evident in conditions of oxygen supply limitation as in extreme hemodilution or significant vasoconstriction (5) where tissue and perivascular venular PO2 are virtually identical.

Hypoxic vasodilation is a physiological response to low tissue oxygen tension in an attempt to match the metabolic demand and delivery of blood. The classical paradigm suggests an oxygen sensor that detects the PO2 in tissue and responds by releasing local mediators such as adenosine, NO, and/or prostacyclin and by causing feed-forward sympathetic activation of β-adrenergic receptors. However, recent publications suggest that the anion nitrite represents a potentially large reservoir of NO, which serves as a critical hypoxic buffer, regulating mitochondrial respiration and hypoxic vasodilation (7, 24).

In conclusion, decreased NO availability magnifies the vasoactive responses of the microcirculation to changes in oxygen supply, reducing the supply to the tissue by increasing oxygen vessel wall consumption. Therefore, impairment of the NO mechanism renders the circulation more sensitive to changes in oxygen availability. Consumption of oxygen by the vessel wall is significant and affects tissue PO2, and it depends on the local PO2. These findings have implications in conditions where NO and oxygen availability decrease at the same time. Furthermore, only normoxia yields a normal FCD, whereas the decrease of NO and vascular oxygen availability compromise capillary perfusion.

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REFERENCES

2. Busse R, Forstermann U, Masuda H, and Pohl U. The role of prosta-
3. Cabrera P, Tsai AG, Frangos JA, and Intaglietta M. Role of endo-
10. Edmunds NJ and Marshall JM. Oxygen delivery and oxygen consump-


