Systemic hypoxia increases leukocyte emigration and vascular permeability in conscious rats

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Wood, John G., Jennifer S. Johnson, Leone F. Mattioli, and Norberto C. Gonzalez. Systemic hypoxia increases leukocyte emigration and vascular permeability in conscious rats. J Appl Physiol 89: 1561–1568, 2000.—We recently observed that acute systemic hypoxia produces rapid increases in leukocyte adherence in the mesenteric microcirculation of the anesthetized rat Wood JG, Johnson JS, Mattioli LF, and Gonzalez NC. J Appl Physiol 87: 1734–1740, 1999; Wood JG, Mattioli LF, and Gonzalez NC. J Appl Physiol 87: 873–881, 1999. Hypoxia-induced leukocyte adherence is associated with an increase in reactive oxygen species (ROS) generation and is attenuated by antioxidants or interventions that increase tissue levels of nitric oxide (NO). These results suggest that the acute effects of hypoxia on leukocyte-endothelial interactions are caused by a change in the ROS-NO balance. The present experiments were designed to extend our observations of the initial microcirculatory response to hypoxia; specifically, we wanted to determine whether the response to systemic hypoxia involves increased microvascular permeability and leukocyte emigration and whether ROS generation and decreased NO levels contribute to these responses. At this time, there is conflicting evidence, from in vitro studies, regarding the effect of hypoxia on these indexes of vascular function. Our studies were carried out in the physiological setting of the conscious animal, in which a prolonged hypoxic exposure is possible without the adverse effects that may develop under anesthesia. The central observation of these studies is that conscious animals exposed for 4 h to environmental hypoxia show increased microvascular permeability and emigration of leukocytes into the extravascular space of the mesenteric circulation. Furthermore, these events are dependent on increased ROS generation and, possibly, a subsequent decrease in tissue NO levels during systemic hypoxia. Our results show that systemic hypoxia profoundly affects vascular endothelial function through changes in the ROS-NO balance in the conscious animal.

mesenteric microcirculation; endothelial cells; venules; nitric oxide donor; leukocyte-endothelial adhesive interactions

WE RECENTLY REPORTED THAT acute systemic hypoxia induced by breathing 10% O2-90% N2 rapidly and markedly increases leukocyte adherence to venular endothelium in the rat mesenteric circulation (28). The increased leukocyte adherence is associated with an increase in reactive oxygen species (ROS) generation (27) and is attenuated by administration of antioxidants (27), nitric oxide (NO) donors, and the NO precursor L-arginine (28). These findings suggest that the acute effects of hypoxia on leukocyte-endothelial interaction are mediated by a change in the ROS-NO balance, which could eventually lead to microvascular damage characterized by increased vascular permeability and emigration of leukocytes into the extravascular space. These latter changes were not observed, however, because our results were obtained in anesthetized animals exposed to systemic hypoxia for a period of only 10 min, a time frame too short to allow for the full manifestation of these responses. Interestingly, rats acclimatized to hypoxia for 3 wk show no evidence of microvascular lesion and are able to tolerate even lower arterial Po2 (PaO2) levels without increases in leukocyte-endothelial interactions (28), implying that the initial microvascular response is eventually resolved during the process of acclimatization. Inhibition of inducible NO synthase in the acclimatized rats resulted in increased adherence of leukocytes to endothelial cells in response to lowered PaO2, suggesting that an upregulation of inducible NO synthase contributes to the acclimatization of the vascular endothelium.

The present experiments were designed to extend our observations of the initial microcirculatory response to hypoxia; specifically, we wanted to determine whether the response to systemic hypoxia involves increased microvascular permeability and leukocyte emigration and whether ROS generation and decreased tissue NO levels contribute to these responses. Available evidence in this respect is controversial. Hypoxia has been reported to either increase (6) or inhibit (21, 25) leukocyte emigration with in vitro preparations; similarly, contradictory results have been reported concerning microvascular permeability (1, 2, 8, 9, 18). We are not aware of studies investigating these phenomena in intact, conscious animals. The present studies were carried out in the physiological setting.
setting of the conscious animal, in which a prolonged hypoxic exposure is possible without the untoward consequences that may develop under anesthesia and that may also allow enough time for leukocyte emigration and increased vascular permeability to be more completely expressed.

METHODS

All surgical and experimental procedures were approved by the Animal Care and Use Committee of the University of Kansas Medical Center. The University of Kansas is accredited by the American Association for the Accreditation of Laboratory Animal Care. Guidelines set by the National Institutes of Health and the Public Health Service Policy on the humane use and care of laboratory animals were followed at all times.

Surgical Preparation

On the day before experiments, male Sprague-Dawley rats (220–300 g) were anesthetized with xylazine-ketamine (2 mg/kg body wt intramuscular), and polyethylene catheters (PE-50) were placed in a jugular vein and a carotid artery. The catheters were tunneled subcutaneously to the back of the neck, exteriorized, cut at a length of 2 in., and flame-sealed. Once surgical procedures were completed, the animals were placed on a heating pad to maintain body temperature during recovery from anesthesia. The rats were then placed in individual cages and given water ad libitum. On the next morning, animals were randomly assigned to one of the experimental protocols.

Intravital Microscopy

During all procedures, the animal’s temperature was maintained at 36–38°C by a homeothermic blanket system (Harvard Apparatus, Natick, MA) connected to an intrarectal temperature probe. Lactated Ringer solution was infused via the jugular catheter at a rate of 2 ml/h, and blood pressure was continuously measured via the carotid artery cannula connected to a digital blood pressure monitor (Micro-Med, Louisville, KY). The abdomen was opened along the midline using a radio-cautery (Harvard Apparatus), and the animal was then positioned on a plexiglass sheet on top of the stage of a Zeiss microscope. The small intestine was carefully removed from the abdomen and positioned over a glass coverslip on a Plexiglas sheet to view the mesenteric microcirculation. The mesentery was covered with a piece of Saran wrap to prevent drying of the tissue and to minimize the effect of ambient oxygen on the mesenteric microcirculation. Mesenteric venules were selected for experiments using the following criteria: 1) straight, unbranched vessels at least 100 μm in length, 2) 20–40 μm in diameter, and 3) no adjacent vessels within 100 μm of the venule. The mesentery was superfused (0.5 ml/min) with phosphate-buffered saline (37°C, pH 7.4) to keep the tissue moist and warm.

Measurement of Leukocyte Emigration Across Mesenteric Venules

Images of mesenteric venules (×40 objective) were recorded on a video cassette recorder with a time-date generator (Panasonic S-VHS) using a Panasonic video camera. Venular diameter was measured using a video caliper (Microcirculation Research Institute, College Station, TX), either on- or off-line, during playback of videotapes. In all experiments, leukocyte emigration was assessed by counting the number of leukocytes in an area defined as 100 μm along the venule by 40 μm away from the vessel surrounding the venules. Emigration was then expressed as the number of extravascular leukocytes per 4 × 103 μm². Five venules were analyzed in each rat, and the results were then averaged to obtain a single estimate of leukocyte emigration for each animal.

Measurement of Vascular Permeability Index

FITC-labeled bovine albumin (50 mg/kg) was intravenously injected in conscious rats ~30 min before examining the mesenteric microcirculation by intravital microscopy. Fluorescence intensity in the microcirculation was recorded using an intensified charge-coupled device camera (C2400, Hamamatsu Photonics, Shizuoku, Japan), with an excitation wavelength of 420–490 nm and an emission wavelength of 520 nm. To minimize photobleaching, the duration of fluorescent recordings was <15 s in a given area of the microcirculation. The fluorescence intensity in three separate venules and surrounding areas was recorded in each rat. After analysis, the three values were averaged to obtain a single estimate for the vascular permeability index in each rat. The first three venules observed that met these criteria were selected for study in each rat.

During playback of videotaped images, fluorescence intensity was measured in three contiguous areas within the venule and three areas in the adjacent perivascular regions using a digital image analysis program (NIH Image 1.62). The intravascular and perivascular areas analyzed were circles with a diameter equal to that of the venule. The values for fluorescence intensity were averaged to obtain a single estimate for intravascular and extravascular fluorescence, and a vascular permeability index was then calculated as the ratio of extravascular to intravascular fluorescence intensities (15).

Measurement of the Dissociation Rate of Spermine NONOate

The dissociation rate of spermine NONOate (SNO) was measured by following changes in absorbance over time, as previously described by Maragos et al. (17). A stock solution of SNO (10⁻² M) was prepared in phosphate-buffered saline and then adjusted to a pH of either 7.4 or 8.5. These stock solutions were then kept at either 25 or 37°C. Every 15 or 30 min, aliquots of the stock solutions were rapidly diluted with phosphate-buffered saline (1:100), and absorbance at 252 nm was then immediately measured.

Experimental Protocols

Series 1: Time course of leukocyte emigration during systemic hypoxia. Conscious rats with the indwelling catheters were placed in a chamber in which 10% O₂ was continuously circulated. The O₂ concentration in the chamber was measured continuously with an Applied Electrochemistry oxygen analyzer. Rats were infused intravenously with saline (2 ml/h) throughout the experiment. After 2 or 4 h of hypoxia, the rats were quickly anesthetized (urethane, 1 g/kg iv via the jugular catheter) and prepared for intravital microscopy. Parallel experiments were conducted in rats treated in the same way but exposed to room air. Intravital microscopy recordings of the mesenteric microcirculation in each rat were made by a person blinded to the experimental protocol.

Series 2: Effect of hypoxia on vascular permeability index. Conscious animals were exposed to hypoxia or normoxia for 4 h, as described in series 1. After ~3.5 h, FITC-albumin was
injected intravenously and the rats were later prepared for intravital microscopy.

Series 3: Effect of antioxidants on hypoxia-induced leukocyte emigration and changes in vascular permeability index. Conscious animals were exposed to hypoxia for 4 h, as described in series 1. In one group, vascular permeability was measured as described, with FITC-albumin injected intravenously ~3.5 h after the animals were placed in the holding chamber. In a second group, leukocyte emigration was measured as described in Measurement of Leukocyte Emigration Across Mesenteric Venules. All animals were given superoxide dismutase (SOD)/catalase (0.5/50 mg/kg iv bolus loading dose) followed by a continuous infusion (1/100 mg·kg⁻¹·h⁻¹) throughout the experiment. After 4 h of hypoxia, the animals were anesthetized and prepared for intravital microscopy.

Series 4: Effect of a nitric oxide donor on hypoxia-induced leukocyte emigration and changes in vascular permeability index. Conscious animals were exposed to hypoxia for 4 h as described in series 1. In one group, vascular permeability was measured, with FITC-albumin injected; 3.5 h after the animals were placed in the holding chamber. In a second group, leukocyte emigration was measured. Both groups of animals were infused with SNO (10⁻² M, pH 8.5, 25°C) throughout the period of hypoxia. Systemic blood pressure was continuously measured, and the infusion rate of SNO was adjusted in each animal throughout the experiment to maintain blood pressure between 85 and 95 mmHg. Infusion rates varied from 0 to 2 ml/h. After 4 h of hypoxia, the animals were anesthetized and prepared for intravital microscopy.

Drugs and Chemicals

Phosphate-buffered saline, BSA, SOD, catalase, and other chemicals were purchased from Sigma Chemical (St. Louis, MO). Spermine NONOate {N-[4-(-1-[3-aminopropyl]-2-hydroxy-2-nitrosohydrazino)butyl]-1,3-propanediamine} was purchased from Cayman Chemical (Ann Arbor, MI). Lidocaine hydrochloride and heparin sodium from porcine intestinal mucosa were purchased from Elkins-Sinn (Cherry Hill, NJ). All solutions were freshly prepared on the day of the experiment.

Statistical Analysis

Means and SEs were calculated for all values from each treatment group. Statistical analysis was carried out by ANOVA. Significance was established using the Bonferroni test for multiple comparisons. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Dissociation Rate of SNO

As shown in Fig. 1, the dissociation rate of SNO was both pH and temperature dependent, also shown by Maragos et al. (17). At pH 7.4 and 37°C, the estimated half-life of SNO was 44.3 min, which is similar to the value of 39 min reported by Maragos et al. (17). The dissociation rate was reduced when the pH was increased to 8.5 and decreased even more when the temperature was lowered from 37°C to 25°C. Under these conditions (pH 8.5 and 25°C), little dissociation of SNO was observed (Fig. 1). These results demonstrate that, during the 4-h period of infusion, the loss of NO activity of the infusate before administration to the animal is negligible. Once the SNO was infused into the circulation, NO release would occur due to the lower pH and the higher body temperature of the animal.

Effect of Hypoxia on Leukocyte Emigration

Few adherent or emigrated leukocytes were noted within the mesenteric microcirculation in animals breathing room air (Fig. 2, left). In contrast, examination of rats that had breathed 10% O₂ for 4 h while conscious (Fig. 2, right) revealed a marked increase in leukocyte emigration. The cumulative data of leukocyte emigration at 2 and 4 h of normoxia and hypoxia are shown in Table 1. Hypoxia produced a statistically significant, time-dependent increase in leukocyte emig-
Systemic hypoxia produced a time-dependent increase in leukocyte emigration across mesenteric venules. This response to hypoxia was significantly attenuated in the groups of rats given either superoxide dismutase (SOD/catalase) or spermine NONOate (SNO). *P < 0.05 vs. corresponding normoxic value. †P < 0.05 vs. corresponding hypoxic value. ‡P < 0.05 vs. corresponding 2-h value.

Table 1. Leukocyte emigration

<table>
<thead>
<tr>
<th>Time</th>
<th>Normoxia</th>
<th>Hypoxia</th>
<th>Hypoxia + SOD/catalase</th>
<th>Hypoxia + SNO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.8 ± 0.8</td>
<td>6.1 ± 1.3*</td>
<td>3.4 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>(n = 5)</td>
<td>(n = 6)</td>
<td>(n = 6)</td>
<td></td>
<td></td>
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<tr>
<td>4 h</td>
<td>2.4 ± 0.4</td>
<td>11.5 ± 1.6‡</td>
<td>3.4 ± 0.6†</td>
<td>5.7 ± 0.7‡†</td>
</tr>
<tr>
<td>(n = 11)</td>
<td>(n = 11)</td>
<td>(n = 7)</td>
<td>(n = 8)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. Systemic hypoxia produced a time-dependent increase in leukocyte emigration across mesenteric venules. This response to hypoxia was significantly attenuated in the groups of rats given either superoxide dismutase (SOD/catalase) or spermine NONOate (SNO). *P < 0.05 vs. corresponding normoxic value. †P < 0.05 vs. corresponding hypoxic value. ‡P < 0.05 vs. corresponding 2-h value.

Table 2 shows values of mean arterial pressure, heart rate, and blood gases at the end of 4 h of normoxia, hypoxia, and hypoxia with continuous infusion of SOD/catalase. Hypoxia produced a significant decrease in arterial blood pressure that was considerably smaller than previously observed in anesthetized rats exposed to this level of hypoxia. Hypoxia also produced the expected increase in heart rate (16) and decreases in PaO₂ and O₂ saturation of Hb. None of these responses to hypoxia were influenced by SOD/catalase administration.
administration. Mean arterial pressure was 87.5 ± 2.8 mmHg after 4 h of hypoxia in the group of rats given SNO, which was significantly lower than the corresponding value in normoxic or hypoxic rats (see Table 3, $P < 0.05$ vs. either group).

**DISCUSSION**

The central observations of these studies are that 1) conscious animals exposed for 4 h to environmental hypoxia show increased microvascular permeability and emigration of leukocytes into the extravascular space of the mesenteric circulation and 2) these events are linked to the generation of ROS during systemic hypoxia. To our knowledge, this study is the first demonstration of leukocyte emigration in any organ during hypoxia in conscious animals. The present observations are consistent with our initial findings during shorter hypoxic exposures in anesthetized rats. These results imply that increased leukocyte adherence to vascular endothelial...
cells is the first step of the microvascular response to hypoxia that is eventually followed by increased permeability and emigration of leukocytes. Accordingly, the phenomena described in conscious animals represent a natural extension of our previous observations in anesthetized animals.

Although hypoxia was interrupted for ~15 min while the rats breathed room air during surgical preparations for intravital microscopy, this is unlikely to have had any effect on the estimated number of leukocytes that emigrated during the previous 4 h of hypoxic exposure. Movement of leukocytes across the vessel wall is a relatively slow process and should not be affected to any appreciable extent during this brief period of normoxia.

The observed changes in cardiovascular function produced by hypoxia were relatively minor compared with those observed in anesthetized rats (28). This indicates that the observed vascular endothelial responses to hypoxia do take place in the physiological setting of the conscious animal. During hypoxia in anesthetized rats, venular shear rate decreases markedly due to the decrease in venular blood flow (28). This is likely due to the accompanying decrease in systemic blood pressure (28) and the sympathetically mediated mesenteric arteriolar vasoconstriction (16). Shear rate is the force generated at the vessel wall that opposes increased leukocyte adherence to the venular endothelium (11). Therefore, a decrease in shear rate could contribute to the initial increase in adhesive interactions and the subsequent emigration of leukocytes. Interventions that block leukocyte adherence, however, do not influence the hypoxia-induced decrease in shear rate, suggesting that this factor cannot solely account for the vascular endothelial response to hypoxia. This conclusion is supported by the findings of this study, which demonstrate that endothelial function was profoundly affected by hypoxia in spite of relatively small changes in systemic blood pressure.

Both the increase in vascular permeability and the leukocyte emigration observed in hypoxia were markedly attenuated by SOD/catalase administration. We previously showed that hypoxia increases ROS generation in mesenteric venules and that antioxidant pretreatment blocks both the ROS generation, as assessed by changes in ROS fluorescence-dependent signal intensity, and the leukocyte-venular adherence (27, 28). Several in vitro studies have reported similar responses: graded hypoxia causes dose-related increases in ROS generation in isolated cardiac myocytes (5, 7) as well as other types of cells (20). An emerging concept in this respect is that reductive stress can result from buildup of reducing equivalents that cannot be transferred to O2 at the mitochondrial cytchrome oxidase in conditions of reduced cellular respiration due to hypoxia (19). Whereas our results strongly support an involvement of ROS in the vascular endothelial response to systemic hypoxia, the site of ROS generation cannot be determined from the available evidence.

The present study shows that administration of an NO donor significantly reduced the degree of both leukocyte emigration and increased vascular permeability that is associated with systemic hypoxia in conscious animals. These results support the concept that the microvascular injury of systemic hypoxia is the result of an alteration in the balance of ROS and NO. Beckman et al. (4) demonstrated that superoxide inactivates NO, resulting in generation of peroxynitrite. In addition to enhanced degradation by ROS, NO levels are also reduced during hypoxia by decreased formation, because oxygen is a substrate for its production. Whorton et al. (26) showed that NO formation in cultured endothelial cells was decreased when the PO2 of the medium was lowered. Regardless of the cause, the potential significance of decreased tissue NO levels is evident from recent studies showing that NO plays a critical role in regulation of the microcirculation. Administration of NO synthase inhibitors promotes leukocyte-endothelial adhesive interactions (14) and leukocyte emigration (12) and increases vascular permeability (3, 13), although opposite results have been reported as well (22). Nonetheless, based on these results, reduced NO levels have been proposed as an initiating event in the pathogenesis of microvascular injury (10).

In the present study, infusion of an NO donor at a rate sufficient to produce a modest but noticeable decrease in mean arterial pressure reduced, but did not totally abolish, the leukocyte emigration and increased vascular permeability of hypoxia. The reason for the incomplete effect could be due to the participation in ROS.

Table 2. *Vascular permeability index*

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Hypoxia</th>
<th>Hypoxia + SOD/Catalase</th>
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<tbody>
<tr>
<td><em>Ratio of Perivascular to Intravascular FITC-Albumin Fluorescence Intensity</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(n – 4)</em></td>
<td><em>(n – 7)</em></td>
<td><em>(n – 6)</em></td>
<td><em>(n – 8)</em></td>
</tr>
<tr>
<td>0.12 ± 0.03</td>
<td>0.99 ± 0.07*</td>
<td>0.25 ± 0.07†</td>
<td>0.56 ± 0.08‡</td>
</tr>
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</table>

Values are means ± SE; n = no. of rats. Hypoxia significantly increased the vascular permeability index in the mesenteric microcirculation. This response involved reactive oxygen species generation as SOD/catalase treatment significantly attenuated hypoxia-induced changes in vascular permeability. In addition, administration of SNO partially reduced the magnitude of this microvascular response to hypoxia. *P < 0.05 vs. normoxia; †P < 0.05 vs. hypoxia.

Table 3. *Arterial blood pressure, heart rate, and arterial blood oxygenation values*

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Hypoxia</th>
<th>Hypoxia + SOD/Catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP, mmHg</td>
<td>129 ± 3</td>
<td>117 ± 4*</td>
<td>116 ± 3</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>335 ± 19</td>
<td>434 ± 15*</td>
<td>406 ± 8</td>
</tr>
<tr>
<td>PaO2, Torr</td>
<td>79 ± 1</td>
<td>40 ± 2*</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>O2 saturation of Hb, %</td>
<td>99.6 ± 0.3</td>
<td>73 ± 3*</td>
<td>72 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Hypoxia produced a significant decrease in mean arterial blood pressure (MABP), arterial PO2 (PaO2), and oxygen saturation of hemoglobin, as well as a significant increase in heart rate (HR). In addition, these responses to hypoxia were not significantly affected by SOD/catalase. *P < 0.05 vs. normoxia.
of other mechanisms in addition to reduced NO levels or to a relatively low dose of NO. Alternatively, the SNO-induced arteriolar dilation could have resulted in an increased venular shear rate, which could, in turn, reduce leukocyte-endothelial interactions and thereby attenuate the microvascular injury. Because NO was infused in conscious animals, its effect on microvascular hemodynamics cannot be determined, as this would require direct observation by intravital microscopy. Nevertheless, the attenuation of the microvascular injury by NO observed in the conscious animal is consistent with our previous observation in anesthetized rats, in which NO attenuated leukocyte-endothelial interactions without influencing the shear rate responses to systemic hypoxia (28).

One interpretation of the effect of antioxidants in our study is that SOD/catalase, by blocking endothelial ROS generation, decreased the adherence of leukocytes to the vascular endothelium and prevented subsequent emigration to the perivascular space. An alternative explanation is that ROS generation results in a chemotactic gradient within the extravascular space. Hypoxia has been shown to increase formation of several proinflammatory mediators from various parenchymal cells, including cardiomyocytes (23) and lung cells (24). Accordingly, SOD/catalase could have prevented the generation of inflammatory mediators that promote leukocyte emigration during hypoxia.

Leukocyte emigration is of pathophysiological significance because emigrated leukocytes can directly cause parenchymal tissue injury through the same processes that result in microvascular damage (10). In addition, diapedesis of leukocytes may disrupt the integrity of the vasculature, resulting in increased vascular permeability, enhanced filtration of plasma into the tissue, and edema formation. These events, if severe enough, may impair delivery of O2 to the tissue and thereby exacerbate organ injury. The results of our study do not indicate to what extent hypoxia-induced changes in vascular permeability are leukocyte-dependent.

Our results differ from those of Mian and Marshall (18), who did not detect extravasation of FITC-albumin during hypoxia in either the mesentery or spinotrapezium of rats. Several differences between their experimental protocol and ours may account for this apparent discrepancy. They utilized a shorter duration of hypoxia (20 min vs. 4 h) in anesthetized, rather than conscious, rats, as in the present study. The brief duration of hypoxia used in their experiment may not have been sufficient for significant extravasation of albumin to occur. In addition, because arterial pressure decreases to a greater extent during hypoxia in anesthetized animals (28) compared with conscious animals (16), intravascular pressures within the mesenteric circulation would likely be lower in the study by Mian and Marshall (18) compared with our present study. Lower intravascular pressures would reduce the rate of albumin extravasation across venules and would confound detection of hypoxia-induced increases in vascular permeability.

In summary, this study shows that 4 h of hypoxia results in a marked increase in vascular permeability and in leukocyte emigration in the mesenteric microcirculation of conscious animals. These changes appear to be dependent on changes in the ROS-NO balance in the microcirculation and represent a natural extension of the increased leukocyte-endothelial adhesive interaction that takes place immediately upon hypoxic exposure. These results are the first demonstration that acute systemic hypoxia profoundly affects vascular endothelial function in the conscious animal.

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