Dissociation between skeletal muscle microvascular PO$_2$ and hypoxia-induced microvascular inflammation

Sidharth Shah, Julie Allen, John G. Wood, and Norberto C. Gonzalez

Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, Kansas 66160-7401

Submitted 23 December 2002; accepted in final form 12 February 2003

Shah, Sidharth, Julie Allen, John G. Wood, and Norberto C. Gonzalez. Dissociation between skeletal muscle microvascular PO$_2$ and hypoxia-induced microvascular inflammation. J Appl Physiol 94: 2323–2329, 2003. First published February 21, 2003; 10.1152/japplphysiol.01185.2002.—Systemic hypoxia (SHx) produces microvascular inflammation in mesenteric, cremasteric, and pial microcirculations. In anesthetized rats, SHx lowers arterial blood pressure (MABP), which may alter microvascular blood flow and microvascular PO$_2$ (PmO$_2$) and influence SHx-induced leukocyte-endothelial adherence (LEA). These experiments attempted to determine the individual contributions of the decreases in PmO$_2$, venular blood flow and shear rate, and MABP to the hypoxia-induced increase in LEA. Cremaster microcirculation of anesthetized rats was visualized by intravital microscopy. PmO$_2$ was measured by a phosphorescence-quenching method. SHx [inspired P O$_2$ of 70 Torr for 10 min, MABP of 65 ± 3 mmHg, arterial P O$_2$ (P$_{aO_2}$) of 33 ± 1 Torr] and cremaster ischemia (MABP of 111 ± 7 mmHg, P$_{aO_2}$ of 86 ± 3 Torr) produced similar PmO$_2$: 7 ± 2 and 6 ± 2 Torr, respectively. However, LEA increased only in SHx (1.9 ± 0.9 vs. 11.2 ± 1.1 leukocytes/100 μm, control vs. SHx, P < 0.05). Phentolamine-induced hypotension (MABP of 55 ± 4 mmHg) in normoxia lowered PmO$_2$ to 26 ± 6 Torr but did not increase LEA. Cremaster equilibration with 95% N$_2$-5% CO$_2$ during air breathing (P$_{aO_2}$(80 ± 1 Torr) lowered PmO$_2$ to 61 ± 1 Torr but did not increase LEA. On the other hand, when cremaster PmO$_2$ was maintained at 60–70 Torr during SHx (P$_{aO_2}$ of 35 ± 1 Torr), LEA increased from 2.1 ± 1.1 to 11.1 ± 1.5 leukocytes/100 μm (P < 0.05). The results show a dissociation between PmO$_2$ and LEA and support the idea that SHx results in the release of a mediator responsible for the inflammatory response.

leukocyte-endothelial interactions; cremaster muscle; microcirculation; ischemia; local hypoxia; tissue PO$_2$

SYSTEMIC HYPOXIA RESULTS in a rapid microvascular inflammatory response characterized by increases in microvascular reactive O$_2$ species (ROS) (19, 20, 24) and in venular leukocyte-endothelial adhesive interactions (26). Rats studied after exposure to 4 h of hypoxia in the conscious state show emigration of leukocytes to the perivascular space and elevated vascular permeability (25). The inflammatory response to hypoxia has been studied predominantly in the mesenteric microcirculation (19–21, 24–26) but has also been observed in venules of the cerebral and cremaster microcirculations (6), indicating that it is a widespread phenomenon. The microvascular lesion eventually resolves; after 3 wk of acclimatization to hypoxia, there is no evidence of leukocyte adherence or emigration in mesentery (26) or cremaster microcirculations (6), and the animals tolerate further reductions in inspired PO$_2$ without evidence of microvascular inflammation.

Although the early response to hypoxia has common features with ischemia-reperfusion, their patterns are quite different and demonstrate that hypoxia and ischemia-reperfusion-induced microvascular inflammation are two distinct phenomena: in hypoxia, ROS increase with the reduction in PO$_2$ and return to normal during normoxic recovery (19–21, 24), whereas in ischemia-reperfusion ROS are low during the ischemic period and increase during reperfusion on reintroduction of O$_2$ (4, 7, 8). Furthermore, leukocyte adherence to the venular endothelium occurs predominantly during reperfusion rather than during ischemia (4, 7, 8, 22, 23), whereas in hypoxia this phenomenon occurs when PO$_2$ is reduced (19, 20, 24); during normoxic recovery, leukocyte-endothelial adhesive interactions actually subside (19, 20, 24). Systemic hypoxia in anesthetized rats, however, results in marked arterial hypotension (26). The decrease in driving pressure tends to reduce venular blood flow, which could enhance leukocyte-endothelial interactions by lowering venular shear rate, i.e., the force generated at the vessel wall by the movement of blood. In fact, systemic hypotension, although more severe and of longer duration than that observed in our studies in systemic hypoxia, has been shown to result in a moderate increase in adherence of leukocytes to skeletal muscle venular endothelium (22). In addition, reduced blood flow will produce a lower microvascular PO$_2$ for any given level of environmental hypoxia. Accordingly, the increased leukocyte-endothelial adherence that accompanies systemic hypoxia could be, at least in part, the result of factors other than the reduced microvascular PO$_2$.

Several interventions that modify the leukocyte-endothelial adhesive interactions of hypoxia do so without altering the hemodynamic response (19, 20, 24–
suggested that hemodynamic changes do not play a major role in the microvascular inflammatory response to hypoxia. Nevertheless, the changes in microvascular blood flow and shear rate that accompany systemic hypoxia in anesthetized rats make it difficult to ascertain to what extent the increase in leukocyte-endothelial adhesive interactions is the direct result of reduced microvascular PO2 and what are the contributions of changes in microvascular hemodynamics. The present experiments were carried out to determine the individual contributions of the decreases in venular shear rate, systemic blood pressure, and microvascular PO2 on the increase in leukocyte-endothelial interactions in skeletal muscle venules that accompanies systemic hypoxia.

METHODS

All procedures were approved by the Animal Care and Use Committee of the University of Kansas Medical Center, an institution accredited by the American Association for the Accreditation of Laboratory Animal Care.

Surgical preparation. Male Sprague-Dawley rats, 175–225 g, were anesthetized with urethane (1.5 g/kg im) after an overnight fast with free access to water. Body temperature was maintained at 36–38°C by use of a homeothermic blanket system connected to an intrarectal temperature probe. PE-50 catheters were inserted in the jugular vein and the carotid artery. Lactated Ringer solution was infused continuously via the jugular vein at a rate of 2 ml/h. Arterial blood pressure was continuously monitored with a digital blood pressure monitor connected to the carotid artery catheter. A tracheotomy was performed, and the trachea was intubated with PE-240 tubing. The tracheal catheter was connected to a rodent nonrebreathing two-way valve, and the animals breathed spontaneously throughout the experiment.

Intravital microscopy. The right cremaster muscle was exposed through a midline scrotal incision as described previously (3). The rat was placed on the platform of a Nikon E600 FN microscope, and the cremaster was spread over a hollow Lucite cylinder, the top of which was sealed with a glass slide. Water was circulated through the cylinder to maintain muscle temperature at 37°C. Muscle temperature was monitored continuously via a thermistor placed underneath the muscle. The cremaster was covered with Saran wrap throughout the experiment.

In experiments in which cremaster microvascular PO2 was altered independently of systemic PO2, the cremaster was spread over a hollow plastic cylinder through which warm, humidified gas of the desired PO2 was circulated and covered by a plastic dome through which the same gas was flushed. The muscle was not covered with Saran wrap in these experiments. Muscle temperature was maintained at 37°C by means of a heating lamp.

Images of the cremasteric microcirculation (×40 objective) were recorded on a videocassette recorder with a time-date generator. Straight, unbranched venules of 100 μm in length and 20–40 μm diameter, with fewer than three adherent leukocytes in a 100-μm segment and no adjacent lymphatics, were selected for microscopic observation. Venular diameter was measured by use of a video caliper. An optical Doppler velocimeter was used to measure venular centerline red blood cell velocity. Average red blood cell velocity was calculated as centerline velocity/1.6 (5). Wall shear rate, which represents the force generated at the vessel wall by the movement of blood, was calculated as 8 × (average red blood cell velocity/venular diameter) (9).

Adhesive interactions of leukocytes with cremasteric venules were assessed off line from videotape playback as follows: rolling leukocytes were defined as those moving along the venular endothelium at a rate lower than red cell velocity. The velocity of rolling leukocytes was calculated by measuring the time it takes a leukocyte to move between two points 100 μm apart along the vessel (10). The total number of rolling leukocytes passing a given point in the vessel was determined in each minute and expressed as the number of leukocytes rolling per minute (rolling leukocyte flux). Adherent leukocytes were defined as those leukocytes that remained stationary for >30 s. Leukocyte adherence was expressed as the number of adherent leukocytes per 100 μm of vessel length.

Measurement of PmO2. A method based on the PO2 dependence of phosphorescence lifetime was used to determine microvascular PO2 (PmO2) (15). The measurement of PmO2 was carried out in separate experiments in which the cremaster was prepared as described above, but the microcirculation was not visualized by using intravital microscopy. The oxyphyr Pd-porphyrin dendrimer (R2) was injected intravenously (16 mg/kg). At this concentration, R2 binds completely to albumin (11); furthermore, R2 has a negative net charge facilitating restriction to the vascular space. Phosphorescence was measured by using a phosphorometer (Oxyspot, Medical Systems, Greenvale, NY) with a bifurcated light guide positioned 2–4 mm above the cremaster. The excitation light emitted from the light guide reached a circular area of the cremaster of ~1 mm diameter and ~500 μm deep. The phosphorescence signal was averaged over 200 ms for each measurement. PmO2 was measured every minute, except during ischemia (see EXPERIMENTAL PROTOCOLS).

The measurement of PmO2 was validated in vitro in the following manner: after administration of R2, rats were exsanguinated under anesthesia, and the blood was equilibrated in a tonometer with gas mixtures of different PO2 values. After equilibration, blood was transferred to glass capillaries, which were sealed with plastic caps and maintained at 37°C. PO2 of the blood in the capillaries was measured by use of the phosphorescence method, after which the blood was introduced anaerobically into a blood-gas electrode assembly and PO2 was measured with a Radiometer blood-gas analyzer.

EXPERIMENTAL PROTOCOLS

In all protocols, ~45 min were allowed for the animals to recover from surgery. The animals breathed spontaneously through a two-way nonrebreathing valve. Approximately half of the experiments were directed to study leukocyte-endothelial interactions via intravital microscopy; in the remaining experiments, PmO2 was measured as described above. Arterial blood samples for measurement of pH, PO2, and PCO2 were obtained at the end of each experimental period.

Systemic hypoxia. These experiments consisted of a 10-min normoxic control period, a 10-min hypoxic period in which the animals spontaneously breathed 10% O2-90% N2, and a 10-min normoxic recovery period. PmO2 was measured every minute during both.

Ischemia. After a 10-min control period, cremaster blood flow was reduced by placing a metal rod over the cremaster pedicle. The weight of the rod decreased average red cell velocity to ~25% of the control value. Ischemia was maintained for 10 min, followed by a 10-min postischemia recov-
ery period. The animals breathed room air throughout the experiment. PmO₂ was measured every minute during the control and recovery periods. During ischemia, PmO₂ was measured at 1, 2, 5, 7, and 10 min after initiation of blood flow occlusion. This lower sampling frequency was used to minimize possible inactivation of the phosphor by repeated excitation during ischemia, when turnover of dye through the circulation at the site of measurement may be restricted by the reduced blood flow, as well as to minimize the possible effects on PmO₂ secondary to photoactivated consumption of O₂ (17). Measurement of PmO₂ of blood contained in glass capillaries every minute for 10 min did not show changes in PmO₂ values as a function of measurement number, indicating that this sampling rate did not introduce systematic measurement errors under these conditions of low blood flow.

Systemic hypotension. After a 10-min control period, phentolamine, an α-adrenoceptor antagonist, was injected (1 mg/kg iv). Phentolamine administration resulted in a rapid decrease in mean arterial blood pressure similar to that seen in systemic hypoxia. The microcirculation was observed for 10 min after the injection of phentolamine. PmO₂ was measured every minute during the control and hypotension periods. Because the hypotension was sustained, no recovery measurements were made.

Cremaster hypoxia, systemic normoxia. In these experiments, the animals breathed air throughout the experiment. The cremaster was equilibrated as described above with a gas mixture of 10% O₂-5% CO₂-85% N₂. After a 10-min control period, local hypoxia of the cremaster was produced by changing the gas mixture equilibrating the muscle to 95% N₂-5% CO₂ while the animal continued breathing room air. After 10 min of cremaster hypoxia, the gas mixture equilibrating the muscle was returned to 10% O₂-5% CO₂-85% N₂.

Cremaster normoxia, systemic hypoxia. In these experiments, the cremaster was equilibrated with a gas mixture of 10% O₂-5% CO₂-85% N₂ throughout the experiment. After a 10-min normoxic control period, the animal breathed 10% O₂-90% N₂, while the muscle continued to be equilibrated with 10% O₂-5% CO₂-85% N₂. The period of systemic hypoxia lasted 10 min and was followed by a 10-min normoxic recovery period.

Statistics. Data are presented as means ± SE. The data after a given treatment were compared with the corresponding pretreatment data by use of a t-test for paired samples. Intergroup comparisons were made with a one-way ANOVA followed by the Bonferroni test for multiple comparisons. A P value of 0.05 or less was considered to indicate a significant difference.

RESULTS

The PmO₂ values obtained by using the phosphorescence quenching method were highly correlated with the values obtained with the PmO₂ electrode in 15 different blood samples (Fig. 1). The slope of the line relating both values was not significantly different from unity, and the intercept was not significantly different from zero.

Figure 2 illustrates the reversible increase in leukocyte-endothelial adherence that accompanies systemic hypoxia: after 10 min of hypoxia, leukocyte-endothelial adherence increased markedly with respect to the normoxic control and returned toward control values 10 min after hypoxia was discontinued.

Systemic hypoxia produced a significant decrease in arterial PmO₂ (Fig. 3A, numerical inset, ○), which resulted in a rapid and reversible decrease in PmO₂ from 35 ± 2 Torr at the end of the normoxic control to 7 ± 2 Torr at 10 min of hypoxia (Fig. 3A, ○, n = 10). The decrease in PmO₂ was accompanied by a decrease in mean arterial blood pressure similar to that seen in systemic hypoxia (Fig. 3C, ○, n = 9). The decrease in shear rate of systemic hypoxia coincided with systemic hypotension and tachycardia (Table 1).

Mechanical restriction of cremaster blood flow produced PmO₂ values that were not different from those seen in systemic hypoxia (Fig. 3A, ●, n = 9); PaO₂ values did not change during the ischemic period (Fig. 3A). Shear rate decreased to levels significantly lower than those seen in systemic hypoxia (Fig. 3B, n = 8), whereas mean arterial blood pressure (MABP) and HR remained unchanged (Table 1). In marked contrast with systemic hypoxia, cremaster ischemia did not result in increased leukocyte-endothelial adherence (Fig. 3C, ●).

Phentolamine decreased MABP to values not different from those seen in systemic hypoxia (Table 1). PaO₂ was not changed by phentolamine (Fig. 3A, numerical insets), whereas PmO₂ showed a modest but significant reduction during hypotension (Fig. 3A, □, n = 5). Phentolamine hypotension was accompanied by a decrease in venular shear rate (Fig. 3B, n = 5) that was not different from that produced during systemic hypoxia. In contrast with hypoxia, leukocyte-endothelial adherence did not increase during hypotension (Fig. 3C).

Figure 4 shows the results of the experiments in which cremaster PmO₂ and arterial PmO₂ were altered independently of one another. Equilibration of the cremaster with 10% O₂-5% CO₂-85% N₂ resulted in average PmO₂ values between 60 and 70 Torr, which were
Fig. 2. Photograph of a cremaster venule during normoxia (A), 10 min after the onset of systemic hypoxia (B), and after 10 min of normoxic recovery (C). Few leukocytes are seen adhering to the endothelium in normoxia. Hypoxia produces a rapid increase in the number of adherent leukocytes that returns toward control after 10 min of normoxic recovery.

Fig. 3. A: time course of microvascular PO2 (PmO2) for the systemic hypoxia (○, n = 10), ischemia (●, n = 9), and hypotension (□, n = 5) experiments. Systemic hypoxia was induced by breathing 10% O2 (inspired PO2 ~70 Torr), ischemia was induced by mechanical obstruction of cremaster blood flow, and hypotension was produced by administration of phentolamine (1 mg/kg iv). Because hypotension was of long duration, no recovery observations were made. Arterial PaO2 data (Torr) are values obtained at the end of the control, experimental, and recovery periods. B and C: data on shear rate and leukocyte-endothelial adherence, respectively, obtained in parallel experiments. The data of B and C were obtained in 10 systemic hypoxia, 9 ischemia, and 5 hypotension experiments. Data are means ± SE.

Neither shear rate (Fig. 4B, n = 8) nor MABP (Table 1) showed significant changes during local cremaster hypoxia. Leukocyte-endothelial adherence remained within control values in spite of the marked reduction in PmO2 (Fig. 4C).

higher than those seen when the animals breathed room air (compare PmO2 control values of Figs. 3A and 4A). Local cremaster hypoxia, in the presence of systemic normoxia (Fig. 4A, ■, n = 8), resulted in a rapid reduction in PmO2 that reached 6 ± 1 Torr after 10 min.
When the cremaster was equilibrated with 10% O₂-5% CO₂-85% N₂ throughout the experiment, Pm O₂ remained at control values even when Pa O₂ was reduced to 35/110 Torr (Fig. 4A, □, n = 7). Shear rate decreased significantly during systemic hypoxia (Fig. 4B, □, n = 6); this coincided with significant hypotension and tachycardia (Table 1). Leukocyte-endothelial adherence increased significantly during systemic hypoxia, in spite of the elevated cremaster PmO₂ (Fig. 4C). The time course and extent of leukocyte-endothelial adherence in this group were not significantly different from those seen in the systemic hypoxia experiments illustrated in Fig. 3C (○) in which cremaster PmO₂ was drastically reduced.

**DISCUSSION**

The main observation of this study is that the increase in leukocyte-endothelial adhesive interactions that occurs during systemic hypoxia may be dissociated from the PO₂ prevalent at the site where the leukocyte adherence takes place. Increased leukocyte adherence occurred during systemic hypoxia whether cremaster PO₂ was reduced or not; conversely, selective reduction of cremaster PO₂ in the presence of systemic normoxia, as evidenced by normal PaO₂ values, did not result in increased leukocyte-endothelial adherence.

Phosphorescence quenching provides a noninvasive measurement of PmO₂ in the microcirculation within the physiological context of the intact animal. The probe binds to albumin and is therefore localized to the plasma phase of the intravascular compartment. In blood, the only molecule that quenches phosphorescence is O₂ (15); thus the lifetime of the phosphorescence originated by excitation of the phosphor is determined by plasma PO₂. The value of PmO₂ obtained in

**Table 1. Mean arterial blood pressure and heart rate values**

<table>
<thead>
<tr>
<th></th>
<th>MABP, mmHg</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systemic hypoxia (n = 18)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>117 ± 8</td>
<td>364 ± 15</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>65 ± 3*</td>
<td>425 ± 8*</td>
</tr>
<tr>
<td>Recovery</td>
<td>115 ± 5</td>
<td>385 ± 12</td>
</tr>
<tr>
<td><strong>Ischemia (n = 17)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>115 ± 6</td>
<td>355 ± 8</td>
</tr>
<tr>
<td>Ischemia</td>
<td>111 ± 7</td>
<td>367 ± 9</td>
</tr>
<tr>
<td>Recovery</td>
<td>118 ± 5</td>
<td>375 ± 12</td>
</tr>
<tr>
<td><strong>Systemic hypotension (n = 10)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>108 ± 2</td>
<td>398 ± 6</td>
</tr>
<tr>
<td>Hypotension</td>
<td>55 ± 4*</td>
<td>475 ± 10*</td>
</tr>
<tr>
<td><strong>Cremaster hypoxia/systemic normoxia (n = 14)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>111 ± 7</td>
<td>388 ± 9</td>
</tr>
<tr>
<td>Cremaster hypoxia</td>
<td>111 ± 8</td>
<td>379 ± 12</td>
</tr>
<tr>
<td>Recovery</td>
<td>109 ± 6</td>
<td>383 ± 8</td>
</tr>
<tr>
<td><strong>Cremaster normoxia/systemic hypoxia (n = 12)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>110 ± 4</td>
<td>375 ± 5</td>
</tr>
<tr>
<td>Systemic hypoxia</td>
<td>68 ± 3*</td>
<td>444 ± 12*</td>
</tr>
<tr>
<td>Recovery</td>
<td>107 ± 6</td>
<td>392 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE. MABP, mean arterial blood pressure; HR, heart rate.

When the cremaster was equilibrated with 10% O₂-5% CO₂-85% N₂ throughout the experiment, PmO₂ remained at control values even when PaO₂ was reduced to 35 ± 1 Torr (Fig. 4A, □, n = 7). Shear rate decreased significantly during systemic hypoxia (Fig. 4B, □, n = 6); this coincided with significant hypotension and tachycardia (Table 1). Leukocyte-endothelial adherence increased significantly during systemic hypoxia, in spite of the elevated cremaster PmO₂ (Fig. 4C). The time course and extent of leukocyte-endothelial adherence in this group were not significantly different from those seen in the systemic hypoxia experiments illustrated in Fig. 3C (○) in which cremaster PmO₂ was drastically reduced.

**DISCUSSION**

The main observation of this study is that the increase in leukocyte-endothelial adhesive interactions that occurs during systemic hypoxia may be dissociated from the PO₂ prevalent at the site where the leukocyte adherence takes place. Increased leukocyte adherence occurred during systemic hypoxia whether cremaster PO₂ was reduced or not; conversely, selective reduction of cremaster PO₂ in the presence of systemic normoxia, as evidenced by normal PaO₂ values, did not result in increased leukocyte-endothelial adherence.

Phosphorescence quenching provides a noninvasive measurement of PmO₂ in the microcirculation within the physiological context of the intact animal. The probe binds to albumin and is therefore localized to the plasma phase of the intravascular compartment. In blood, the only molecule that quenches phosphorescence is O₂ (15); thus the lifetime of the phosphorescence originated by excitation of the phosphor is determined by plasma PO₂. The value of PmO₂ obtained in

![Fig. 4. A: Time course of microvascular PO₂ (PmO₂) in experiments in which the cremaster muscle was maintained in normoxic conditions while the animal breathed 10% O₂ (cremaster normoxia/syste mic hypoxia, □, n = 7) and experiments in which the cremaster underwent 10 min of hypoxia while the animal remained normoxic throughout the experiment (cremaster hypoxia/systemic normoxia, ■, n = 8). B and C: data on shear rate and leukocyte-endothelial adherence, respectively, obtained in parallel experiments. The data of B and C were obtained in 6 cremaster normoxia/systemic hypoxia experiments and 6 cremaster hypoxia/systemic normoxia experiments. Data are means ± SE.](http://jap.physiology.org/)

Downloaded from http://jap.physiology.org/ by 10.220.33.5 on July 12, 2017
these experiments is the result of a signal originating in the blood contained in the tissue sampled; accordingly, it is a weighted average determined by the relative proportion of the blood contained in the arterioles, capillaries, and venules. Although the method as such does not provide an exact value of Po2 in each of these vascular segments, it certainly provides a reliable estimate of the state of oxygenation of the tissue under study, because PmO2 reflects the balance between the rates of O2 delivery and consumption of the tissue sampled (14).

Leukocyte-endothelial interactions follow a coordinated series of steps initiated by rolling and margination of leukocytes, followed by increased vascular permeability and emigration of leukocytes to the perivascular space (7). These processes are mediated through specific adhesion molecules expressed on the surface of leukocytes and endothelial cells. Margination and rolling of leukocytes is mediated by selectins, which are expressed both in leukocytes and endothelial cells, whereas firm adherence of leukocytes to the endothelium is mediated by integrins (4, 7). In the present experiments, the initial response, increased leukocyte-endothelial adherence, was used as an early index of hypoxia-induced inflammation.

The results of these studies indicate that the reduction in shear rate that accompanies systemic hypoxia in anesthetized rats does not play a role in the increased leukocyte adherence observed in the experimental conditions of these experiments because decreasing shear rate either via systemic hypotension or local ischemia was not followed by increased leukocyte-endothelial adherence. These results support previous observations in the mesentery showing that antioxidants and exogenous NO prevent hypoxia-induced increased leukocyte-endothelial adherence without altering the effect of hypoxia on shear rate (19, 20, 24–26).

Although PmO2 was reduced to essentially the same levels in ischemia and in systemic hypoxia, increased leukocyte-endothelial adherence occurred only in the latter (Fig. 3, A and C). A possible explanation for this discrepancy is that the reduction of blood flow of ischemia is accompanied by additional effects, for example, a reduction in tissue pH and an increase in Pco2, and that these or other ischemia-associated effects could influence the expression of adhesion molecules and offset the effects of reduced PmO2. We think that this explanation is unlikely on the basis of the experiments that show that selective cremaster hypoxia, in the presence of systemic normoxia and unchanged cremaster blood flow, also fails to elicit increased leukocyte-endothelial adherence.

A second possible explanation for the lack of effect of local hypoxia on leukocyte-endothelial adherence is that the time spent by leukocytes in the hypoxic cremaster is too short for complete leukocyte activation. This could also apply to the ischemic cremaster in which blood red cell velocity was reduced to ~25% of the control value.

However, if the lack of increase in leukocyte-endothelial adherence during local cremaster hypoxia or ischemia were exclusively due to insufficient time of exposure to the reduced Po2, the fact that leukocytes adhered to the endothelium of the normoxic cremaster during systemic hypoxia (Fig. 4, A and C) would mean that only leukocytes, and not endothelial cells, need to be exposed to hypoxia for leukocyte-endothelial adherence to develop. Expression of adhesion molecules increases when isolated leukocytes are exposed to hypoxia (13, 16, 18), although it is not clear whether in this case leukocytes would adhere to normoxic endothelium. Experiments in vitro studying the adhesive interactions of leukocytes and endothelial cells when Po2 of each cell type is altered independently could provide information on this possibility.

The combined data presented in this study, namely that leukocyte adherence to cremaster venular endothelium increases only during systemic hypoxia, independent of cremaster PmO2, could also be explained by the release of a mediator triggered by hypoxia acting at some central site such as the lungs. The local microvascular response to this hypothetical mediator would be independent of the PmO2 prevailing at that site. This possibility is supported by the observation that maintaining a relatively high PmO2 level did not prevent leukocyte adherence when the animals breathed 10% O2 (Fig. 4, A and C). If the inflammatory response to hypoxia were mediated through an agent acting at distant sites, the rapid and widespread nature of the microvascular response suggests that such an agent would be a substance already present or rapidly synthesized at some central site such as the lungs. The stimulus for the release of the hypothetical mediator would be the decrease in Po2 at some specific site, and not systemic hypotension, because a reduction in blood pressure under normoxic conditions did not lead to increased leukocyte-endothelial adhesive interactions (Fig. 3C, Table 1).

We do not think that there is a conflict between the idea of a mediator and the numerous observations showing increases in leukocyte-endothelial interactions in response to hypoxia under in vitro conditions (1, 2, 12, 13, 16, 18). The in vivo response to hypoxia is undoubtedly complex, and it is possible that different mechanisms may come into play at different times. Although the present results do not entirely rule out a role of local Po2, they are consistent with the notion of a mediator released by hypoxia from a central site. This interpretation helps provide a plausible explanation for the initial microvascular changes elicited by systemic hypoxia in the intact animal. It is clear that further research is necessary to rule out other possible explanations and to determine the existence and nature of such an agent, as well as the possible mechanisms responsible for its synthesis and release.

In summary, the present study demonstrates a dissociation between PmO2 and hypoxia-induced increased leukocyte-endothelial adhesive interactions in skeletal muscle. Although other possible mechanisms are not totally ruled out, the results are consistent with the notion that the microvascular response to hypoxia is
mediated through an agent released in response to the low systemic PO2.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-39443 (to N. C. Gonzalez) and HL-64195 (to J. G. Wood).

REFERENCES


