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Dissociation between skeletal muscle microvascular P_{O_2} and hypoxia-induced microvascular inflammation

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Shah, Sidharth, Julie Allen, John G. Wood, and Norberto C. Gonzalez. Dissociation between skeletal muscle microvascular P_{O_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 P_{O_2} (Pm_{O_2}) and influence SHx-induced leukocyte-endothelial adherence (LEA). These experiments attempted to determine the individual contributions of the decreases in Pm_{O_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. Pm_{O_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} (Pa_{O_2}) of 33 ± 1 Torr] and cremaster ischemia (MABP of 111 ± 7 mmHg, Pa_{O_2} of 86 ± 3 Torr) produced similar Pm_{O_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 Pm_{O_2} to 26 ± 6 Torr but did not increase LEA. Cremaster equilibration with 95% N_2-5% CO_2 during air breathing (Pa_{O_2} of 80 ± 1 Torr) lowered Pm_{O_2} to 6 ± 1 Torr but did not increase LEA. On the other hand, when cremaster Pm_{O_2} was maintained at 60–70 Torr during SHx (Pa_{O_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 Pm_{O_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 P_{O_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 P_{O_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 P_{O_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 P_{O_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 P_{O_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 P_{O_2}.

Several interventions that modify the leukocyte-endothelial adhesive interactions of hypoxia do so without altering the hemodynamic response (19, 20, 24–

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26), suggesting 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 P_{O_2} 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 P_{O_2} 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 P_{O_2} was altered independently of systemic P_{O_2}, the cremaster was spread over a hollow plastic cylinder through which warm, humidified gas of the desired P_{O_2} 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 Pm_{O_2}. A method based on the P_{O_2} dependence of phosphorescence lifetime was used to determine microvascular P_{O_2} (Pm_{O_2}) (15). The measurement of Pm_{O_2} 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 oxyphor 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. Pm_{O_2} was measured every minute, except during ischemia (see EXPERIMENTAL PROTOCOLS).

The measurement of Pm_{O_2} 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 P_{O_2} values. After equilibration, blood was transferred to glass capillaries, which were sealed with plastic caps and maintained at 37°C. P_{O_2} 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 P_{O_2} 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, Pm_{O_2} was measured as described above. Arterial blood samples for measurement of pH, P_{O_2}, and P_{CO_2} were obtained at the end of each experimental period.

Systemic hypoxia. These experiments consisted of a 10-min normoxic control period, a 10-min hypoxia period in which the animals spontaneously breathed 10% O_2-90% N_2, and a 10-min normoxic recovery period. Pm_{O_2} was measured every minute.

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-

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ery period. The animals breathed room air throughout the experiment. Pm_{O_2} was measured every minute during the control and recovery periods. During ischemia, Pm_{O_2} 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 Pm_{O_2} secondary to photoactivated consumption of O_2 (17). Measurement of P_{O_2} of blood contained in glass capillaries every minute for 10 min did not show changes in P_{O_2} 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. Pm_{O_2} 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% O_2-5% CO_2-85% N_2. After a 10-min control period, local hypoxia of the cremaster was produced by changing the gas mixture equilibrating the muscle to 95% N_2-5% CO_2, while the animal continued breathing room air. After 10 min of cremaster hypoxia, the gas mixture equilibrating the muscle was returned to 10% O_2-5% CO_2-85% N_2.

Cremaster normoxia, systemic hypoxia. In these experiments, the cremaster was equilibrated with a gas mixture of 10% O_2-5% CO_2-85% N_2 throughout the experiment. After a 10-min normoxic control period, the animal breathed 10% O_2-90% N_2, while the muscle continued to be equilibrated with 10% O_2-5% CO_2-85% N_2. 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 P_{O_2} values obtained by using the phosphorescence quenching method were highly correlated with the values obtained with the P_{O_2} 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 P_{O_2} (Fig. 3*A*, numerical inset, ○), which resulted in a rapid and reversible decrease in Pm_{O_2} from 35 ± 2 Torr at the end of the normoxic control to 7 ± 2 Torr at 10 min of hypoxia (Fig. 3*A*, ○, n = 10). The decrease in Pm_{O_2} was accompanied by a decrease in shear rate (Fig. 3*B*, ○, n = 9) and a marked and reversible increase in leukocyte endothelial adherence (Fig. 3*C*, ○, n = 9). The decrease in shear rate of systemic hypoxia coincided with systemic hypotension and tachycardia (Table 1).

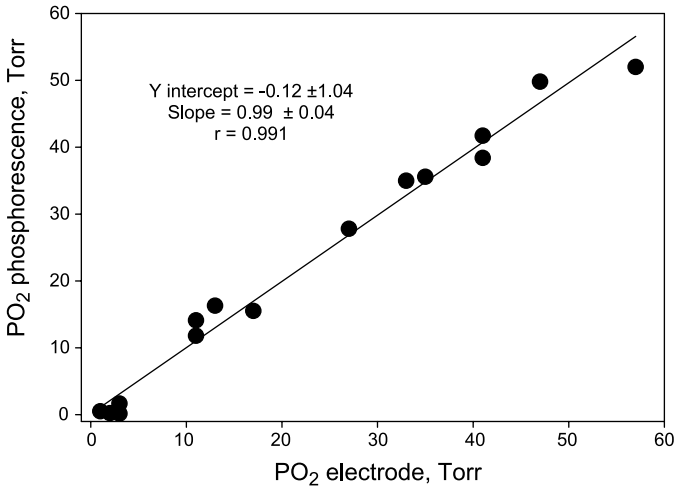

Fig. 1. P_{O_2} values obtained by using the phosphorescence quenching technique plotted as a function of the P_{O_2} value obtained by using a conventional P_{O_2} electrode on the same blood sample. Each point represents an individual blood sample. The intercept is not significantly different from zero, and the slope is not significantly different from unity.

Mechanical restriction of cremaster blood flow produced Pm_{O_2} values that were not different from those seen in systemic hypoxia (Fig. 3*A*, ●, n = 9); Pa_{O_2} values did not change during the ischemic period (Fig. 3*A*). Shear rate decreased to levels significantly lower than those seen in systemic hypoxia (Fig. 3*B*, 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. 3*C*, ●).

Phentolamine decreased MABP to values not different from those seen in systemic hypoxia (Table 1). Pa_{O_2} was not changed by phentolamine (Fig. 3*A*, numerical insets), whereas Pm_{O_2} showed a modest but significant reduction during hypotension (Fig. 3*A*, □, n = 5). Phentolamine hypotension was accompanied by a decrease in venular shear rate (Fig. 3*B*, 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. 3*C*).

Figure 4 shows the results of the experiments in which cremaster Pm_{O_2} and arterial P_{O_2} were altered independently of one another. Equilibration of the cremaster with 10% O_2-5% CO_2-85% N_2 resulted in average Pm_{O_2} values between 60 and 70 Torr, which were

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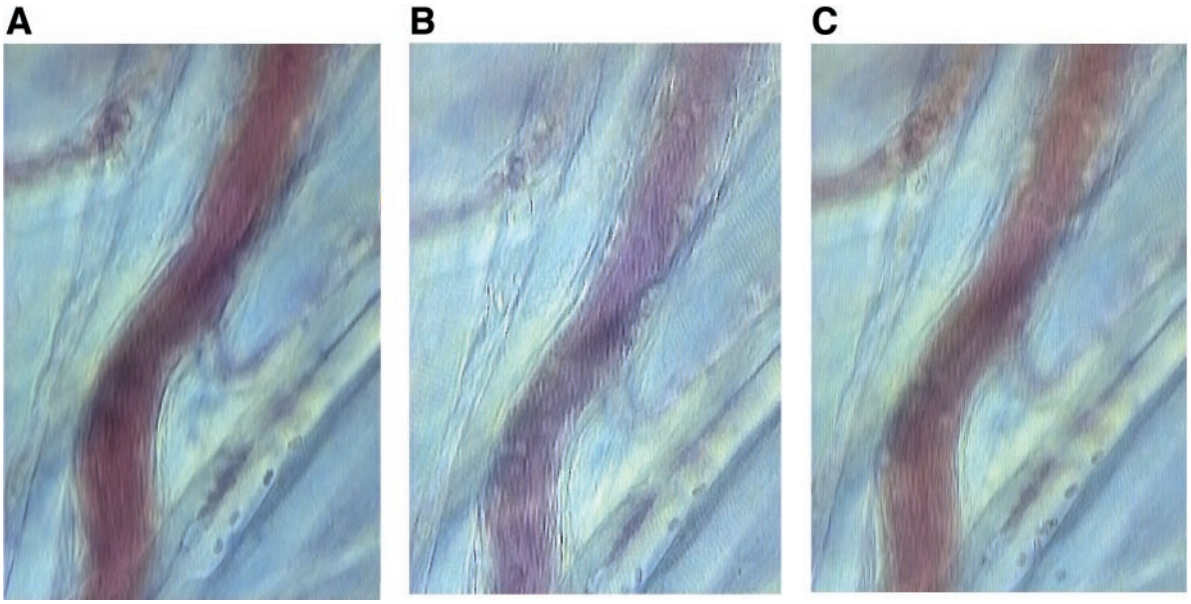

Fig. 2. Photograph of a cremaster venule during normoxia (*A*), 10 min after the onset of systemic hypoxia (*B*), and after 10 min of normoxic recovery (*C*). Few leukocytes are seen adhering to the endothelium in normoxia. Hypoxia produces a rapid increase in the number of adherent leukocytes that returns toward control after 10 min of normoxic recovery.

higher than those seen when the animals breathed room air (compare Pm_{O_2} control values of Figs. 3*A* and 4*A*). Local cremaster hypoxia, in the presence of systemic normoxia (Fig. 4*A*, ■, $n = 8$), resulted in a rapid reduction in Pm_{O_2} that reached 6 ± 1 Torr after 10 min. Neither shear rate (Fig. 4*B*, $n = 8$) 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 Pm_{O_2} (Fig. 4*C*).

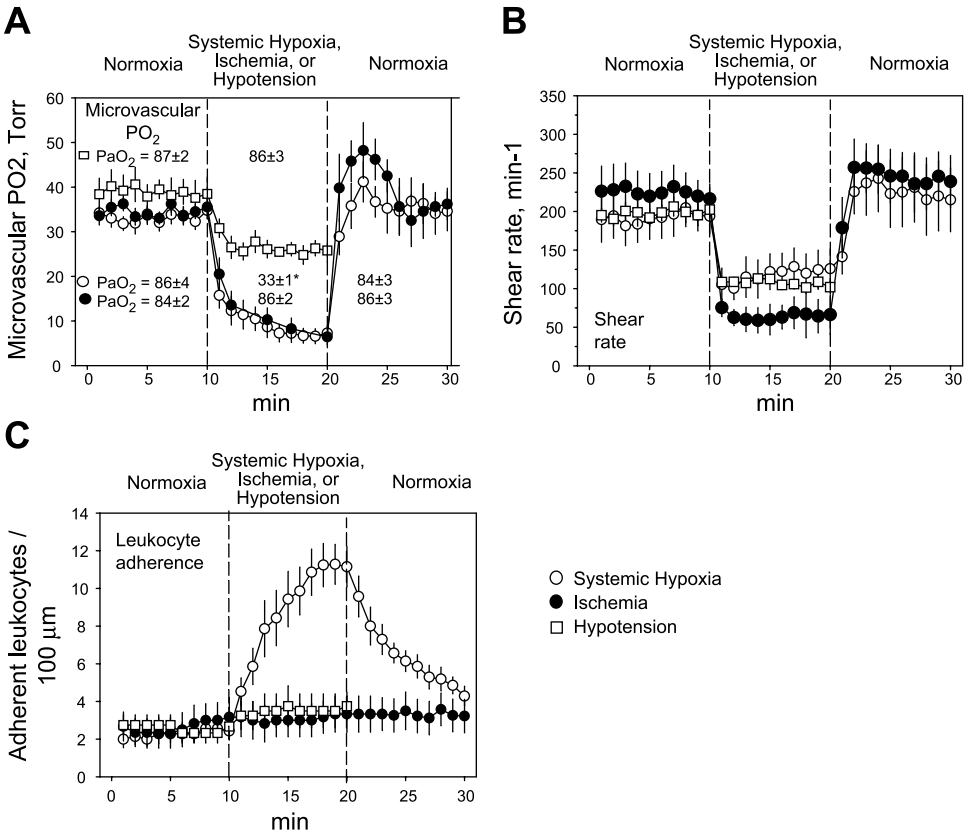

Fig. 3. *A*: time course of microvascular P_{O_2} (Pm_{O_2}) for the systemic hypoxia (○, $n = 10$), ischemia (●, $n = 9$), and hypotension (□, $n = 5$) experiments. Systemic hypoxia was induced by breathing 10% O_2 (inspired P_{O_2} ~70 Torr), ischemia was induced by mechanical obstruction of cremaster 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 Pa_{O_2} 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.

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

	MABP, mmHg	HR, beats/min
Systemic hypoxia (n = 18)		
Control	117 ± 8	364 ± 15
Hypoxia	65 ± 3*	425 ± 8*
Recovery	115 ± 5	385 ± 12
Ischemia (n = 17)		
Control	115 ± 6	355 ± 8
Ischemia	111 ± 7	367 ± 9
Recovery	118 ± 5	375 ± 12
Systemic hypotension (n = 10)		
Control	108 ± 2	398 ± 6
Hypotension	55 ± 4*	475 ± 10*
Cremaster hypoxia/systemic normoxia (n = 14)		
Control	111 ± 7	388 ± 9
Cremaster hypoxia	111 ± 8	379 ± 12
Recovery	109 ± 6	383 ± 8
Cremaster normoxia/ systemic hypoxia (n = 13)		
Control	110 ± 4	375 ± 5
Systemic hypoxia	68 ± 3*	444 ± 12*
Recovery	107 ± 6	392 ± 6

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

When the cremaster was equilibrated with 10% O_2-5% CO_2-85% N_2 throughout the experiment, Pm_{O_2} remained at control values even when Pa_{O_2} was reduced to 35 ± 1 Torr (Fig. 4*A*, □, n = 7). Shear rate decreased significantly during systemic hypoxia (Fig. 4*B*, □, 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 Pm_{O_2} (Fig. 4*C*). 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. 3*C* (○) in which cremaster Pm_{O_2} 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 P_{O_2} prevalent at the site where the leukocyte adherence takes place. Increased leukocyte adherence occurred during systemic hypoxia whether cremaster P_{O_2} was reduced or not; conversely, selective reduction of cremaster P_{O_2} in the presence of systemic normoxia, as evidenced by normal Pa_{O_2} values, did not result in increased leukocyte-endothelial adherence.

Phosphorescence quenching provides a noninvasive measurement of Pm_{O_2} 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 O_2 (15); thus the lifetime of the phosphorescence originated by excitation of the phosphor is determined by plasma P_{O_2}. The value of Pm_{O_2} obtained in

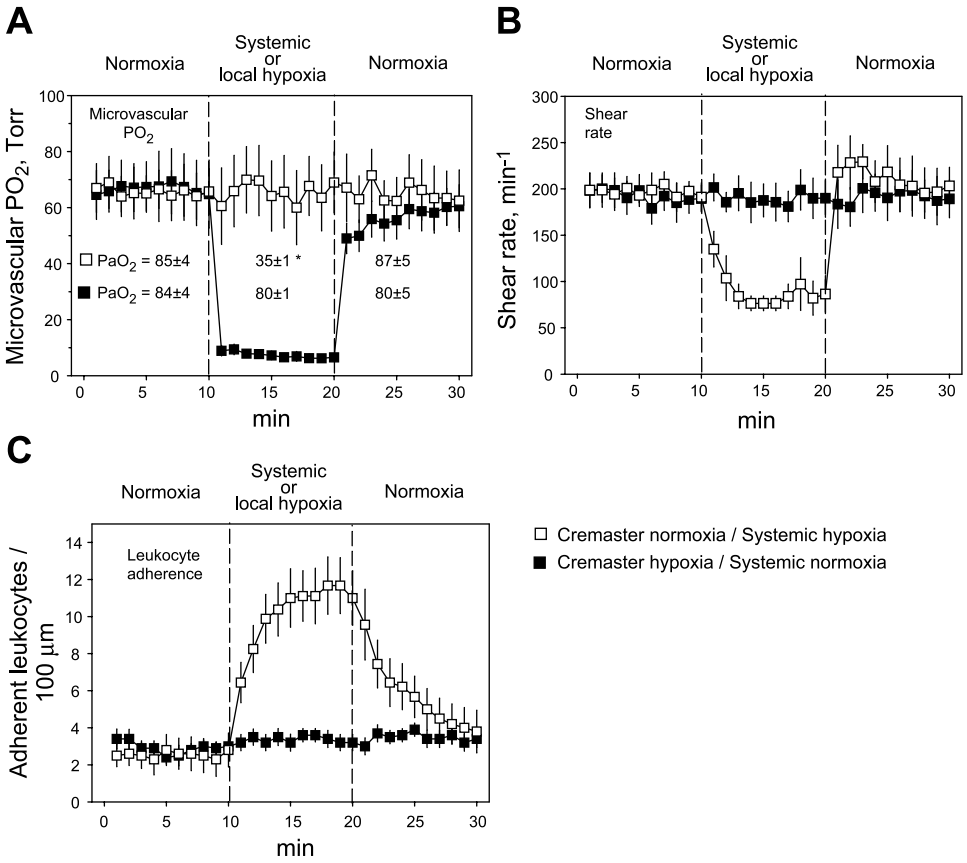

Fig. 4. *A*: Time course of microvascular P_{O_2} (Pm_{O_2}) in experiments in which the cremaster muscle was maintained in normoxic conditions while the animal breathed 10% O_2 (cremaster normoxia/ systemic hypoxia, □, n = 7) and experiments in which the cremaster underwent 10 min of hypoxia while the 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.

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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 P_{O_2} in each of these vascular segments, it certainly provides a reliable estimate of the state of oxygenation of the tissue under study, because Pm_{O_2} reflects the balance between the rates of O_2 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 Pm_{O_2} 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 P_{CO_2}, and that these or other ischemia-associated effects could influence the expression of adhesion molecules and offset the effects of reduced Pm_{O_2}. 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 P_{O_2}, 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 P_{O_2} 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 Pm_{O_2}, 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 Pm_{O_2} prevailing at that site. This possibility is supported by the observation that maintaining a relatively high Pm_{O_2} level did not prevent leukocyte adherence when the animals breathed 10% O_2 (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 P_{O_2} 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. 3*C*, 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 P_{O_2}, 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 Pm_{O_2} 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

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mediated through an agent released in response to the low systemic P_{O_2}.

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