Effect of acute hypoxia on microcirculatory and tissue oxygen levels in rat cremaster muscle

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Johnson, Paul C., Kim Vandegriff, Amy G. Tsai, and Marcos Intaglietta. Effect of acute hypoxia on microcirculatory and tissue oxygen levels in rat cremaster muscle. J Appl Physiol 98: 1177–1184, 2005; doi:10.1152/japplphysiol.00591.2004.—Repeated exposure to brief periods of hypoxia leads to pathophysiological changes in experimental animals similar to those seen in sleep apnea. To determine the effects of such exposure on oxygen levels in vivo, we used an optical method to measure PO2 in microcirculatory vessels and tissue of the rat cremaster muscle during a 1-min step reduction of inspired oxygen fraction from 0.21 to 0.07. Under control conditions, PO2 was 98.1 ± 1.9 Torr in arterial blood, 52.2 ± 2.8 Torr in 29.0 ± 2.7-μm arterioles, 26.8 ± 1.7 Torr in the tissue interstitium near venous capillaries, and 35.1 ± 2.6 Torr in 29.7 ± 1.9-μm venules. The initial fall in PO2 during hypoxia was significantly greater in arterial blood, being 93% complete in the first 10 s, whereas it was 68% complete in arterioles, 47% at the tissue sites, and 38% in venules. In the 10- to 30-s period, the fall in normalized tissue and venular PO2 was significantly greater than in arterial PO2. At the end of hypoxic exposure, PO2 at all measurement sites had fallen very nearly in proportion to that in the inspired gas, but tissue oxygen levels did not reach critical PO2. Significant differences in oxyhemoglobin desaturation rate were also observed between arterial and microcirculatory vessels during hypoxia. In conclusion, the fall in microcirculatory and tissue oxygen levels in resting skeletal muscle is significantly slower than in arterial blood during a step reduction to an inspired oxygen fraction of 0.07, and tissue PO2 does not reach anaerobic levels.

IN RECENT YEARS, THE PHYSIOLOGICAL and pathological effects of brief periods of hypoxia (≤1 min) have come under increasing scrutiny due to the growing interest in sleep apnea. Animal studies have demonstrated an acute increase in sympathetic nerve activity (12) and local vasodilator mechanisms (6, 22) during short periods of hypoxia. With chronic intermittent hypoxia over a period of weeks, experimental animals exhibit sustained elevation of sympathetic nerve activity and arterial pressure and a decrease in cardiac function (2, 12, 20, 30). Similar changes in blood pressure and cardiac function have been seen in patients with sleep apnea (15).

As the responses to brief periods of hypoxia have become increasingly well documented, there is a need for information on the changes in oxygen levels in the organism that elicit these responses. Studies in animals have described the time course of arterial desaturation during induced apnea (13, 39), whereas studies in humans have documented the changes in arterial blood saturation during voluntary apnea (11) or sleep apnea (5). However, information on the time course and magnitude of changes in oxygen tension at the microcirculatory and tissue levels is lacking. Such information would be useful in assessing the effects of arterial desaturation on microcirculatory PO2 and oxyhemoglobin (HbO2) and on tissue PO2 levels during episodes of sleep apnea.

Using a 1-min exposure of anesthetized rats to 7% O2 in N2, we examined the rate and magnitude of the fall in PO2 in arterial blood, the microcirculation, and tissue sites at the venous end of the capillary network where tissue PO2 is believed to be lowest (23). This drop in inspired oxygen fraction (FIO2) is at least as large as would be expected in sleep apnea (5, 13). We tested the hypothesis that the time course of change in PO2 and HbO2 saturation in microcirculatory vessels and in tissue PO2 during hypoxia is slower than in arterial blood. We also tested the hypothesis that the tissue PO2 during hypoxia would fall into the range where a shift from aerobic to anaerobic metabolism is known to occur.

METHODS

Animal preparation. Studies were performed on 33 fasted male Sprague-Dawley rats (165 ± 2 g body wt) anesthetized with pentobarbital sodium (60 mg/kg, Nembutal, Abbott Laboratories) administered by intraperitoneal injection. Supplemental anesthesia consisting of Nembutal was administered through a catheter in the right jugular vein to maintain a surgical level of anesthesia as determined by appropriate criteria. The studies were approved by the University of California, San Diego Animal Subjects Committee. The right carotid artery was cannulated to obtain arterial blood samples and to measure systemic arterial pressure. A tracheal tube was inserted to maintain a patent airway. In the microcirculatory studies, the cremaster muscle was prepared in a manner similar to that described in a previous report (7) based on the original description by Baez (1). During surgery, the muscle was continuously irrigated with physiological salt solution and subsequently covered with polyvinyl film (Saran Wrap), and the muscle and animal were placed on a heated Lucite platform. The animal was then mounted on a microscope stage for viewing and measurement of microcirculatory variables and oxygen levels. A 30-min period of equilibration on the stage was allowed before the first protocol was initiated.

Systemic measurements. Arterial pressure was measured with a Becton Dickinson pressure transducer (model DTX Plus TNF-R) and recorded on a Biopac model MP150CE (Biopac Systems, Santa Barbara, CA). Arterial Pco2 and Po2 during the control and experimental periods were measured on 100-μl blood samples taken from the carotid artery using a model 248 CHIRON Diagnostics (Halstead, UK) blood gas analyzer.

Microscope and oxygen measurement systems. The microscope system used in these studies has been used previously for in vivo determination of oxygen levels in microcirculatory vessels and parenchymal tissue (35). The system has the capability for repeated mea-
measure of oxygen tension at localized sites and measurement of vessel dimensions and red cell velocity of selected vessels. Briefly, the system consists of an inverted microscope IMT2 (Olympus, New Hyde Park, NY), including a Hg arc for transillumination of the tissue and a video camera and tape recorder for monitoring and recording the visual field. A strobe light source and photomultiplier tube are incorporated to measure the time decay of phosphorescence of an oxygen-sensitive dye. A ×20 objective (numerical aperture = 0.46, Olympus) was used to obtain a final magnification of 180 μm on the horizontal axis and 140 μm on the vertical axis of the video monitor.

Because the microscopic field could not be visualized during oxygen measurements, data on vessel diameter and flow were obtained only during the control period. Diameter measurements were taken from the video image using a Digital Video Image Shearing Monitor model 908 (Vista Electronics, San Diego, CA). Velocity measurements were made using a Fiber Optic Photo Diode Pickup and Velocity Tracker model 102B (Vista Electronics) and corrected to mean red cell velocity as described previously (35).

To determine oxygen levels, the microscope system includes a model 6601 strobe light source (EG & G, Salem, MA) for epillumination and excitation of a phosphorescent dye, a photomultiplier tube and amplifier for recording the phosphorescence decay, and custom software for calculating the mean PO2 and standard deviation of the measurement. The method of PO2 measurement was developed first by Vanderkooi et al. (38) and adapted in our laboratories for microcirculatory studies (16, 35). The method has been used by a number of other laboratories to measure PO2 in microcirculatory vessels as reviewed in Ref. 36. In respect to measurements in tissue where dye concentration is lower than blood, simultaneous PO2 measurements were made in tissue with our system and the Whalen oxygen system consists of an inverted microscope IMT2 (Olympus, New Hyde Park, NY), including a Hg arc for transillumination of the tissue and a video camera and tape recorder for monitoring and recording the visual field. A strobe light source and photomultiplier tube are incorporated to measure the time decay of phosphorescence of an oxygen-sensitive dye. A ×20 objective (numerical aperture = 0.46, Olympus) was used to obtain a final magnification of 180 μm on the horizontal axis and 140 μm on the vertical axis of the video monitor.

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Procedure for microcirculatory oxygen measurement. To measure oxygen tension in the microcirculation and tissue, 1D-meso-tetra(4-carboxyphenyl)porphine (Porphyrin Products, Logan, UT) was prepared as described previously (25) and injected intravenously at a dose of 15 mg/kg body wt. The dye distributes rapidly in the bloodstream and diffuses into the extracellular tissue spaces in sufficient concentration to allow measurement of oxygen levels in interstitial fluid as well as microcirculatory vessels. Phosphorescence was excited by the strobe light source that delivered a total of 10 flashes at 244 Hz. Measurements were made in tissue with our system and the Whalen oxygen microelectrode showed no significant difference between the two methods (36).

To estimate the HbO2 saturation from PO2 measurements, the characteristic HbO2 saturation curve for rats of this strain (Sprague-Dawley) and supplier (Simonson, Gilroy, CA) was determined as described previously (37). The PO2 necessary to obtain 50% oxygen saturation (PO250) was 32.4 Torr and Hill’s n50 was 2.61 (n = 2). These values are similar to those found for albino rats of 35.4 ± 1.3 and 2.56 ± 0.09 (means ± SD), respectively, by Teissier et al. (33). The latter investigators obtained a value of 0.56 for the Bohr affect, which was used in this study to correct for pH effects on the PO2-HbO2 relationship. The pH of arteriolar, venous capillary, and venular blood was assumed to be 0.13 unit less than arterial blood based on the study of Kobayashi and Takizawa (18). It was assumed that the pH change in microcirculatory vessels during hypoxia was the same as that in arterial blood. HbO2 saturation was determined from the P50 and n50 values using Hill’s equation.

Hypoxia system. The hypoxia system consists of parallel circuits for directing either air or 7% oxygen in nitrogen through a T tube attached to the tracheal catheter. Rapid switching was achieved by means of a manually controlled three-way valve. Flowmeters allowed adjustment of the flow rate to 2 l/min for both gases. In several experiments, a FOXY spectrophotometer with AL300 probe (Ocean Optics, Dunedin, FL) was used to monitor the time course of change in oxygen concentration at the entrance to the tracheal cannula.

Exposure protocol. Hypoxia was induced by switching from air to the 7% oxygen mixture for a period of 1 min. We selected a 1-min period of exposure to fully cover the range of change in oxygen levels expected to occur in voluntary or sleep apnea (5, 27). In the group used for arterial blood-gas determinations, blood samples were taken while the animal breathed room air, beginning at 7, 27, or 54 s after switching to 7% oxygen and at the end of the recovery period. Arterial blood samples required a period of 6 s. To minimize blood loss from repetitive sampling, the recovery value after a hypoxic period was taken as the control for the subsequent period if that value was not different from the preceding control value.

In the animals used for microscopic determinations of oxygen tension, arterial pressure was recorded along with the oxygen level at the chosen site (an arteriole, venule, or tissue site at the venous end of the capillary network). During a 1-min control period, three oxygen measurements were obtained at 0, 30, and 60 s followed by measurements at 10, 30, and 60 s of hypoxia after which ventilation with air was restored. Measurements were continued at 30-s intervals for 2 min into the recovery period. After an intervening period of −10 min, the protocol was repeated at a different site.

Capillary PO2 could not be measured directly due to low signal level with our system in vessels this size. To obtain an estimate of capillary PO2 for the purpose of evaluating capillary HbO2 saturation, we first determined the difference between the PO2 in postcapillary venules (<13-μm inner diameter), where the signal level was sufficient, and in adjacent tissue sites. Measurements were made during a control period, after 1 min of exposure to 10% oxygen, and after 2 min of recovery. These data were then used together with the PO2 in tissue sites near the capillaries to estimate capillary PO2 and HbO2 saturation as described in RESULTS.

Statistics and data analysis. All values are presented as means ± SE. The statistical significance of changes in arteriolar, venular, and tissue PO2 during the experimental procedure was determined by the repeated-measures one-way ANOVA followed by the Student-Newman-Kuels test. Comparisons between groups and between arterial blood values at different time points utilized a one-way ANOVA followed by the Student-Newman-Kuels test. A P value of <0.05 was considered significant. The coefficient of variation (CV; standard deviation/mean) was calculated for PO2 values obtained from arteriole, venular, and tissue sites for each time point in the study. To determine the CV of the measurement procedures, repeated determinations of arterial blood PO2 were made on a single arterial blood sample and repeated determinations with the phosphorescence technique on a single blood sample in a microhemocritor tube. The CV for arterial blood PO2 with the CHIRON blood gas-analyzer was 1.3% (n = 10) and 2.1% for PO2 with the phosphorescence technique (n = 10). A two-way ANOVA was used to compare the CV in the microcirculation and tissue during control and hypoxia.

RESULTS

Hemodynamic measurements. Mean arterial pressure in the control period averaged 130 ± 2.4 mmHg. Mean red cell velocity in the control period averaged 8.0 ± 0.99 mm/s in arterioles and 2.2 ± 0.60 mm/s in venules. Arterial pressure fell to 81 ± 5 mmHg at 1 min of hypoxia and returned to 129 ± 4 mmHg 2 min after PO2 returned to 0.21.

Effect of hypoxia on inspired and arterial blood-gas levels. When FIO2 was dropped from 0.21 to 0.07, the change at the inlet to the tracheal catheter, as determined with the Ocean Optics FOXY spectrophotometer, was 95% complete in 2.6 ± 0.12 s (time constant 0.61 ± 0.05 s) (n = 5). The return to 0.21 was 95% complete in 3.2 ± 0.18 s (time constant = 0.82 ±
from each other. Tissue PO2 near the venous capillaries fell to

PO2 dropped rapidly with 68% of the change to the final value

change occurring in the first 10 s. PO2 values in venules at all

10-s value (P < 0.001) but not significantly different from
each other. Note that most of the fall in arterial PCO2 occurred

in the first 10 s.

Arterial blood pH rose from 7.41 ± 0.01 in the control period to 7.51 ± 0.02 after 1 min and returned to control levels in the recovery period. The 10-s, 30-s, and 1-min values were significantly different from control (P < 0.001) but not from each other.

Effect of hypoxia on microcirculatory and tissue PO2. The time course of change in microcirculatory oxygen levels during hypoxia is shown in Fig. 1. Under control conditions, PO2 values were stable. The mean PO2 during the control period was 52.2 ± 2.8 Torr in 29.0 ± 2.7-μm arterioles (n = 12), 35.1 ± 2.6 Torr in 29.7 ± 1.9-μm venules (n = 10), and 26.8 ± 1.7 Torr in the tissue interstitium near venous capillaries (n = 11). All of these values were significantly different from each other (P < 0.05).

When the hypoxic gas mixture was introduced, arteriolar PO2 dropped rapidly with 68% of the change to the final value of 15.8 ± 1.2 Torr occurring in the first 10 s. All hypoxia values were significantly less than control (P < 0.001). The 30- and 60-s values in arterioles were significantly lower than the 10-s value (P < 0.001) but not from each other. Venular PO2 reached a final value of 11.4 ± 1.0 Torr with 38% of the change occurring in the first 10 s. PO2 values in venules at all time points during hypoxia were significantly lower than control (P < 0.001). PO2 levels in venules at 30 and 60 s were significantly different from that at 10 s (P < 0.001) but not from each other. Tissue PO2 near the venous capillaries fell to 9.6 ± 1.0 Torr at 60 s with 47% of the change occurring in the first 10 s. All hypoxia values in tissue were significantly less than control (P < 0.001). The 30- and 60-s values in tissue were significantly lower than the 10-s value (P < 0.001) but not from each other. When PO2 was returned to 0.21, the PO2 at arteriolar and tissue sites returned to a stable value within 30 s, whereas the mean venular PO2 appeared to lag somewhat; it was significantly different from the subsequent recovery values (P < 0.01). To summarize, during hypoxia, PO2 in the microcirculation and tissue fell significantly from control to 10 s and from 10 to 30 s but not between 30 and 60 s. We note, however, that during the latter period the mean PO2 in all three groups dropped (Fig. 1) and PO2 decreased at 29 of the 33 measurement sites.

Heterogeneity of PO2 in the microcirculation and tissue increased during hypoxia. The mean CV rose significantly from 22% in the control period to 29% during hypoxia but was not significantly different among microcirculatory vessels and tissue. The increased CV during hypoxia was not due to variation in arterial PO2 among animals as the latter was 9% in the control period and 8% during hypoxia. As noted in METHODS, the CV of the measurement procedures was much lower than the in vivo values, namely 1.3% for arterial blood gas and 2.1% for the phosphorescence technique.

Comparison of arterial, microcirculatory, and tissue oxygen tension changes during hypoxia. PO2 levels in microvascular and tissue compartments are compared with arterial PO2 in Fig. 2. The control value of arterial PO2 was significantly greater than microcirculatory and tissue values and remained significantly above those levels throughout hypoxic exposure as shown in Fig. 2A. Similarly, microcirculatory and tissue values were significantly different from each other except for arterioles vs. venules at 10 s and venules vs. tissue at 60 s. In the first 10 s, the absolute change in arterial and arteriolar PO2 was significantly greater than in venules and tissue, and the fall in arterial PO2 was significantly more than arteriolar PO2. Between 10 and 30 s and between 30 and 60 s there were no significant differences in the change in PO2 among compartments, although, as noted above, microcirculatory and tissue PO2 did fall

Table 1. Arterial blood gas and pH during hypoxia and recovery

<table>
<thead>
<tr>
<th>Time</th>
<th>PO2, Torr</th>
<th>PCO2, Torr</th>
<th>pH</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>98.0 ± 1.9</td>
<td>39.2 ± 0.9</td>
<td>7.41 ± 0.01</td>
<td>16</td>
</tr>
<tr>
<td>10 s</td>
<td>36.5 ± 1.4</td>
<td>24.7 ± 2.2</td>
<td>7.54 ± 0.02</td>
<td>6</td>
</tr>
<tr>
<td>30 s</td>
<td>32.7 ± 1.7</td>
<td>21.7 ± 1.0</td>
<td>7.57 ± 0.01</td>
<td>5</td>
</tr>
<tr>
<td>60 s</td>
<td>31.7 ± 0.9</td>
<td>27.2 ± 1.0</td>
<td>7.51 ± 0.02</td>
<td>5</td>
</tr>
<tr>
<td>Recovery</td>
<td>95.4 ± 2.3</td>
<td>37.3 ± 1.9</td>
<td>7.42 ± 0.20</td>
<td>15</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals.
significantly between 10 and 30 s, whereas arterial PO2 did not. The normalized changes for all compartments are shown in Fig. 2. After 60 s of hypoxia, the percent changes are very similar in all compartments, ranging from 64 to 68%, and there are no significant differences among sites. However, the changes during earlier time periods are quite different. PO2 in the arterial blood fell 63% below the control value in the first 10 s, which is not significantly different from the 47% fall in the arterioles but is significantly different from that in the tissue spaces (30%; \( P < 0.01 \)) and the venules (23%; \( P < 0.001 \)). The change in arteriolar PO2 is also significantly greater than in tissue and venules (\( P < 0.05 \) and 0.01, respectively). In the interval from 10 to 30 s, the pattern is reversed with normalized changes in venular and tissue significantly greater than in arterial blood (\( P < 0.01 \) and 0.05, respectively), whereas the drop in venular PO2 is significantly greater than arteriolar PO2 (\( P < 0.05 \)). Between 30 and 60 s, there is no change in arterial oxygen level, whereas there appears to be a downward trend in microcirculatory and tissue values that, as noted above, is not significant. The normalized rate of change in PO2 for each compartment is presented in Table 2. It is evident that between control and 10 s the rate of change is successively slower in each of the more distal compartments, whereas in the subsequent periods the trend is toward a more rapid change in the more distal compartments, reflecting the fact that the change is not complete in those areas.

Effect of hypoxia on longitudinal PO2 differences between compartments. The sequential changes in oxygen levels as blood transits the circulatory system are shown in Fig. 3. In this graph, it is apparent that, under control conditions, most of the PO2 drop occurs between the arterial and arteriolar sites, i.e., 45.9 compared with 25.4 Torr between arterioles and tissue adjacent to venous capillaries. There is a rise of 8.3 Torr from tissue to the venules, indicating an overall drop of 17.1 Torr from 29-Torr arterioles to venules of similar size. After 10 s of hypoxia, the drop from the arterioles to tissue is virtually

![Fig. 2. A: PO2 levels in arterial blood, arterioles, venules, and tissue sites in the venous capillary network during a 1-min reduction of FIO2 from 0.21 to 0.07 and a subsequent return to 0.21. B: percent changes in the PO2 levels shown in A. Data points are means ± SE.](image1)

![Fig. 3. Longitudinal profile of PO2 levels in arterial, arteriolar, and venular blood and tissue sites in the venous capillary network during reduction of FIO2 from 0.21 to 0.07 for 1 min and return to 0.21. Data points are means ± SE.](image2)

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**Table 2. Normalized PO2 rate**

<table>
<thead>
<tr>
<th>Site</th>
<th>Control to 10 s</th>
<th>10–30 s</th>
<th>30–60 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>6.24±0.16</td>
<td>0.20±0.14</td>
<td>0.03±0.03</td>
</tr>
<tr>
<td>Arteriole</td>
<td>4.75±0.36</td>
<td>0.86±0.16</td>
<td>0.12±0.06</td>
</tr>
<tr>
<td>Tissue</td>
<td>3.01±0.56</td>
<td>1.36±0.19</td>
<td>0.22±0.09</td>
</tr>
<tr>
<td>Venule</td>
<td>2.32±0.70</td>
<td>1.69±0.34</td>
<td>0.32±0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE.
Changes in HbO2 saturation during hypoxia. The estimated HbO2 saturation in the large artery and microcirculatory vessels during hypoxia is shown in Fig. 4 and was obtained from the PO2 data shown above and the P50 and n50 values given in METHODS using Hill’s equation. Corrections for pH are as described in METHODS.

Venous capillary HbO2 saturation was estimated using the tissue PO2 value (Fig. 2) obtained in the venous capillary region. To test the assumption that this tissue PO2 can be used as an estimate of capillary PO2, we measured the difference in PO2 between postcapillary venules (inner diameter of 10.4 ± 0.62 μm) and adjacent tissue (n = 5) during control, 1-min exposure to 10% oxygen in the inspired air, and recovery. The mean PO2 in the postcapillary venules was 0.56 ± 0.56 Torr higher than in adjacent tissue, but the two values were not significantly different. Therefore, it was concluded that the tissue PO2 values from Fig. 2 could be used as an estimate of PO2 in the adjacent venous capillaries.

Under control conditions, the arterial blood is ~95% saturated, and arteriolar blood is 69% saturated. A somewhat larger difference (39%) is found between arterioles and venous capillaries with an increase of ~16% from that site to the venules.

Overall, there is a difference of 50% in HbO2 saturation from artery to 30-μm venules. All HbO2 saturation values during the control period were significantly different from each other. At 10 s into the hypoxic period, the HbO2 saturation in the arterioles has fallen by about the same amount as in arterial blood, but the drop in the capillaries and venules is considerably less. As a consequence, the overall difference in HbO2 saturation from artery to venule is now 32%. At 10 s, all HbO2 saturation values are significantly different from each other except for arterioles vs. venules.

At 30 and 60 s of hypoxia, the overall arteriovenous difference in HbO2 saturation returns to the control level (50%), but almost all of the oxygen loss now occurs in the arterial to arteriolar section. Arterial saturation at 30 and 60 s is significantly greater than microcirculatory levels. Arteriolar HbO2 saturation is significantly greater than capillary and venular levels at 30 and 60 s, but the latter two compartments are not significantly different. It is also notable that at 60 s there is little oxygen left in the blood arriving at the venous capillaries and venules; the estimated HbO2 saturation in these compartments is 5 and 6%, respectively.

The rate of change of HbO2 saturation in the first 10 s (% saturation/s) was significantly greater in arterial blood than in venules and in arterioles compared with both capillaries and venules, but there were no significant differences in the subsequent periods. In contrast, the normalized rate was not significantly different among the compartments in the first 10 s of hypoxia, whereas in the 10- to 30-s period, the rate of desaturation was significantly greater in capillary and venular blood compared with arterial blood and in venular compared with arteriolar blood but not between arterial and arteriolar blood.

DISCUSSION

Systemic effects of hypoxia. Exposure to 7% oxygen for 1 min led to a substantial (40%) decrease in arterial pressure in this study. A similar finding has been reported previously in rats during hypoxia (9, 28) and has been attributed to a dominance of local vasodilator mechanisms in the peripheral circulation over centrally mediated vasoconstrictor mechanisms (9). However, we found in a separate study (unpublished results) using our hypoxic regimen that blood flow in the cremaster muscle falls to a greater degree than arterial pressure, suggesting that vasoconstriction may predominate in this muscle.

Causal factors in arterial blood desaturation. In this study, exposure to 7% oxygen in nitrogen led to a rapid drop in PO2, at the entrance to the tracheal cannula that was 95% complete in 2.6 ± 0.12 s. The subsequent fall in arterial PO2 was 93% complete at the time of our first measurement in hypoxia (10 s). Mean arterial PO2 values at 30 and 60 s are slightly lower than at 10 s, but the differences were not statistically significant. Studies of the dynamics of arterial desaturation during apnea have led to the suggestion that the process occurs in two stages, the first being due to depletion of the oxygen store in the lung and the second due to depletion of oxygen in the blood (39). It was also shown that the oxygen level in the venous blood was an important determinant of the magnitude of the initial change. In the present study, depletion of the oxygen store in the lung would have occurred very rapidly with the shift in
inspired oxygen level. That may primarily account for the fall in the arterial blood PO2 at 10 s. The venous oxygen level, as reflected in venular PO2, continued to fall throughout the hypoxic period, but depletion of this source apparently was not sufficient to cause a significant fall in the oxygen level of the arterial blood.

Oxygen levels in the circulation in the control state. Under control conditions, the PO2 in arterial blood was 98 Torr and 52 Torr in 29-μm arterioles, as shown in Fig. 2. A substantial PO2 gradient along the arteriolar network has been reported previously by a number of investigators (4, 8, 17, 34). Studies in this laboratory have shown, in addition, a large PO2 gradient across the arteriolar wall, leading to the suggestion that the longitudinal gradient is mainly due to a high rate of oxygen consumption of the arteriolar wall (34). The further fall to 27 Torr at venous capillary tissue sites presumably reflects additional loss from the more distal arterioles and the capillary network. The small rise in PO2 of 8 Torr from tissue sites to 30-μm venules is consistent with the findings of others (16, 29, 31) and may be due to mixing of well-oxygenated blood from high flow pathways with poorly oxygenated blood from low flow pathways (31). It may also reflect diffusive shunting of oxygen from arterioles to adjacent venules as seen in this muscle between paired small arteries and veins (19).

PO2 changes in the circulation with acute hypoxia. In support of our first hypothesis, this study demonstrates a progressively slower fall in PO2 in the more distal regions of the circulation compared with arterial blood during acute hypoxia. In the first 10 s, the change in arterial PO2 was significantly greater than microcirculatory and tissue PO2, whereas arteriolar PO2 fell more than venular and tissue PO2. PO2 in arterial blood reached 93% of its final value, whereas the PO2 change was 68% complete in 29-μm arterioles, 47% at venous capillary tissue sites, and 38% in 30-μm venules. In the remaining time periods, there were no significant differences among compartments in absolute changes in PO2, although between 10 and 30 s PO2 did fall significantly in the microcirculation and tissue but not in arterial blood. Between 30 and 60 s, there was a downward trend in mean PO2 of the microcirculation and tissue, but it was not statistically significant.

The slower time course of change in PO2 in tissue and microcirculatory vessels compared with arterial blood is also evident in Fig. 2B, in which normalized (percent) changes are plotted. During the first 10 s, the normalized change of arterial PO2 was significantly greater than that in venules and tissue but not compared with arterioles. Between 10 and 30 s, the trend reversed with the normalized change being significantly greater in tissue and venules compared with arterial blood and in venules compared with arterioles. The rate of change in PO2 for the four compartments during the three time periods presented in Table 2 shows a slower change in the more distal compartments during the first 10 s and a subsequent reversal of that trend since the PO2 in the more distal compartments continues to fall proportionately more than in the arterial compartment. This reflects the overall time course of fall in PO2 during hypoxia, which was significantly longer in venules and tissue compared with arterial blood.

The slower changes in the microcirculation and tissue may reflect the time required for the deoxygenated blood to reach the microcirculation as well as the oxygen storage capacity of the tissue. The mean transit time of labeled red blood cells and plasma in the rat cremaster muscle has been reported to be ~5 s from artery to 28-μm arterioles and ~10 s from artery to 32-μm venules (3). These values are highly dependent on the state of vascular tone and would increase with a higher degree of tone, for example. The oxygen storage of the tissue can be estimated from the solubility of oxygen in muscle tissue (0.047 ml oxygen·ml tissue−1·atm−1 at 35°C) (14). If the average tissue PO2 falls by the same amount as in our measurement site (8.0 Torr in the first 10 s), the tissue would supply 0.00049 ml oxygen/ml tissue or 35% of the oxygen consumption of 0.79 ml·min−1·100 g−1 (0.85 ml·min−1·100 ml−1 assuming a specific gravity of 1.08) reported in another rodent muscle (hamster retractor) (10). An alternative calculation of oxygen consumption is based on the maximal rate of fall in tissue PO2 of 1.44 Torr/s obtained at a tissue site 30 μm from a 20-μm venule when blood flow to the rat spinotrapezius muscle was occluded (25). This estimate is based on the maximal slope shown in Fig. 2 of that study. This fall is presumably due to oxygen consumption, and an 8-Torr drop would provide 52% of the oxygen consumption of the tissue. Between 10 and 30 s of hypoxia, the contribution of tissue oxygen stores to oxygen consumption would be half as great and in the last 30 s would be <5%. Because the PO2 values used in these calculations were from the tissue area where PO2 is lowest, these values likely underestimate the actual contribution. From these observations, it appears that both the blood transit time and the oxygen content of the tissues contribute significantly to the slower fall in microcirculatory and tissue PO2 compared with arterial blood during brief hypoxia.

Changes in microvascular PO2 at 1 min of hypoxia. As shown in Fig. 2B, at 1 min of hypoxia, the fall in PO2 in arterial blood, the microcirculation and tissue are all very nearly in proportion to the drop in FIO2 (67%). Similarly, Shah et al. (28) found that, after a 1-min exposure to 10% FIO2, the mean PO2 of a 1-mm-diameter region of the rat cremaster muscle determined with a similar technique fell to the same degree as the inspired gas. Figures 1 and 2 show that, in our studies, the absolute drop in PO2 during hypoxia was much greater in the arterioles than in the other microvascular compartments, and as a consequence the difference between the arterioles and the tissue and venules decreased. In addition, at 1 min of hypoxia, the rise in PO2 from the tissue capillary site to the venules was much smaller than in the control state. In agreement with this finding, Stein et al. (30) found higher oxygen levels in the larger venules of the hamster retractor muscle compared with the small venules during normoxic breathing but not in hypoxia. This finding could be explained if the higher oxygen levels in the larger venules were due to uneven oxygen extraction at the capillary level in the control state and the site of major oxygen extraction shifted upstream to the arteriolar network in hypoxia. Alternatively, because the PO2 difference between arterioles and venules also decreased greatly in our study, there would be less countercurrent exchange between such vessels lying adjacent to each other, as has been shown in this muscle from small arteries adjacent to small veins (19).

The CV of PO2 in the microcirculation and tissue under control conditions was substantially higher than in the arterial blood, as would be expected due to heterogeneity of microcirculatory flow. With hypoxia, the CV of microcirculatory and tissue PO2, but not of arterial blood PO2, rose significantly. This observation does not support the hypothesis that the PO2
HbO2 saturation with hypoxia. In this study, we found that the mean PO2 in postcapillary venules (10.4 ± 0.6-μm inner diameter) is 0.46 ± 0.56 Torr higher than that in adjacent tissue, but the difference is not statistically significant. Based on this finding, we made the assumption that the PO2 in capillary blood can be considered equivalent to that of adjacent tissue for purposes of calculating HbO2 saturation.

The changes in HbO2 saturation in the blood during hypoxia may be understood by reference to Figs. 3 and 4. During the control period, HbO2 saturation drops by a little more than 60% from the artery to venous capillaries, with a small increase from these vessels to the 30-μm venules. After 10 s of hypoxia, arterial and arteriolar HbO2 saturation have fallen by similar amounts, although the fall in PO2 in the arterioles is much less than in the arteries (32 vs. 62 Torr), reflecting the steeper slope of the HbO2 dissociation curve at lower PO2. The degree of desaturation is also influenced by the leftward shift of the dissociation curve with a shift in arterial pH, which is already apparent with 10 s of hypoxia. This change is obviously secondary to hyperventilation since PCO2 falls (Table 1) and tends to maintain a higher HbO2 saturation than if ventilation were constant. At the capillary and venule level, the estimated drop in HbO2 is small due to the very small change in PO2 (9 Torr). At 30 s of hypoxia, there is little further change in arterial HbO2 saturation but large changes in HbO2 in arterioles and venules due to the greater changes in PO2 and the steep slope of the dissociation curve at these PO2 levels. Between 30 and 60 s, there is no further change in arterial PO2 and HbO2 and minor changes in estimated HbO2 in the microvasculature with the small additional drop in PO2.

HbO2 saturation data also support our first hypothesis. During the first 10 s, the rate of change in HbO2 saturation was significantly greater in the arterial compartment than in venules and arterioles compared with both capillaries and venules. When the data were normalized, differences were seen only in the 10- to 30-s period, where a significant downward trend was seen in capillaries and venules compared with arterial blood and in venules compared with arterioles. Compared with the PO2 data, there were fewer instances of significant differences in the change in HbO2 saturation due to the steeper slope of the dissociation curve in the microcirculatory vessels offsetting, in part, the smaller change in PO2.

The fall in arterial HbO2 of 40% at 1 min of hypoxia should lead to similar shifts in the microcirculatory compartments if oxygen delivery along that pathway remained constant. In fact, the venular HbO2 saturation also fell 40% at 1 min, but the change was much greater in the arterioles, indicating that most of the oxygen loss at this time occurred upstream from these 29-μm vessels. It is possible that the loss upstream reflects slower blood flow in the arterioles, which would lead to a larger fraction of the total oxygen loss in these vessels. As noted above, we have observed that microcirculatory flow decreases more than the fall in arterial pressure during this hypoxic protocol (unpublished results). On the basis of this observation, it would be expected that the overall difference in HbO2 saturation would increase during hypoxia. This was not seen for reasons that are not clear. In estimating the change in HbO2 saturation in the microcirculation during hypoxia, we have assumed that the pH shift is equivalent to that in the arterial blood. If the shift is less, HbO2 saturation in the microvascular vessels during hypoxia would be lower than we have calculated.

Changes in tissue PO2 during hypoxia. The tissue PO2 values we obtained in the control state (26.8 ± 1.7 Torr) are in the upper part of the range found previously in this muscle; with the use of oxygen microelectrodes, mean values of 19 and 30 Torr were reported in two studies of tissue PO2 (21, 24). A value of 22.8 ± 3.3 Torr was found in the vicinity of venous capillaries of cat sartorius muscle using oxygen microelectrodes (4). In rat spinotrapezius muscle, the PO2 obtained with the phosphorescence technique at tissue sites 30 μm from a 20-μm venule was 17.1 ± 0.5 Torr (25).

In the rat spinotrapezius muscle, tissue levels of NADH began to rise when interstitial PO2 measured by the phosphorescence technique fell to 2.4–2.9 Torr (25). In the present study, the mean interstitial PO2 in the vicinity of venous capillaries was 9.6 ± 1.0 Torr at the end of the 60-s hypoxic exposure and the two lowest individual values were 5.4 and 5.5 Torr. Based on an earlier study (23), the venous capillary network is the region where a shift to anaerobic metabolism would first occur. Therefore, the findings of this study do not support our hypothesis that during this hypoxic regimen tissue PO2 would fall to a level at which a shift to anaerobic metabolism is known to occur. We note, however, that the variability in PO2 (CV) in the microcirculation and tissue was somewhat greater during hypoxia than in the control state, increasing the likelihood that some tissue areas would become hypoxic, although none were seen in this study. It should also be noted that our control tissue PO2 values were near the high end of the range previously reported and may reflect a lower level of arteriolar vascular tone (36), although mean red cell velocity in the arterioles (8.0 ± 0.99 mm/s) in our study appears to be about one-third less than that found in another study on vessels of similar size in the same muscle (21). Under conditions of higher vascular tone or any other condition that would decrease the ratio of oxygen delivery to oxygen consumption, the critical PO2 could be reached. Intracellular acidosis as determined with 31P-NMR developed in the rat brain when arterial PO2 was reduced to ~41 Torr (26).

Possible relation to sleep apnea. Our findings may provide some insight to the changes in oxygen tension in microcirculatory vessels and tissue during sleep apnea. In that disorder, the mean duration of apnea is 21–24 s, according to clinical reports (5, 27), and thus would fall within the time frame where the greatest change occurred in our study. A study by Fletcher et al. (13) showed that arterial oxygen saturation fell 25.5% over a 30-s period during obstructive apnea in baboons, whereas in our study estimated arterial saturation decreased 31% in this same time period. This suggests that with obstructive apnea of 30-s duration the fall in PO2 levels in the microcirculation and tissues would be slightly less than those observed in our study, at least in vascular beds with similar ratios of blood flow to oxygen consumption. Although the tissue interstitial PO2 with 30-s hypoxia is considerably lower than seen under control conditions, it is still well above the threshold for a shift to anaerobic metabolism. It appears, therefore, that episodes of sleep apnea are not likely to induce anaerobic metabolism in resting muscle, although the increased
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heterogeneity of microcirculatory Po2 with hypoxia makes it difficult to rule this out completely. Our finding that the change in Po2 in the microcirculation and tissue at equilibrium is very nearly proportional to the change in inspired gas may be useful in interpreting changes in sleep apnea. In the latter case, the rate of change of the alveolar gas and arterial blood is slower than in this study, and as a consequence it is likely that the time lag between Po2 changes in the arterial blood and the microcirculation in sleep apnea is much less than with a step change.

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