ABSTRACT

The changes in microvascular diameter and perivascular oxygen tension resulting from alterations in suffusion solution Po$_2$ were investigated in a study of the participation of oxygen in the regulation of blood flow. Diffusion gradients for oxygen were altered by changing the Po$_2$ of a solution covering the surface of the hamster cheek pouch. As the solution Po$_2$ was raised from a low of 11 mm Hg, perivascular Po$_2$ of the large arterioles initially decreased to a minimum at approximately 40 mm Hg and then increased progressively as solution Po$_2$ was elevated further. Arterial capillary and tissue Po$_2$ remained relatively constant over a range of solution oxygen tensions between 11 and 40 mm Hg, suggesting that either the precapillary sphincters or the terminal arterioles were active in regulating tissue Po$_2$ as the input of O$_2$ from the solution was increased. The arterioles constricted as solution Po$_2$ was elevated. Average arteriolar diameter decreased by 13% as solution Po$_2$ increased from 11 to 47 mm Hg. A more pronounced constriction of 20% occurred when solution Po$_2$ was increased from 11 to 84 mm Hg. These experiments indicated that the response of large and small arterioles was not mediated by a direct effect of oxygen on the vascular smooth muscle, since decreases in perivascular oxygen tension were coincident with decreases in vascular diameter in these vessels over a range of solution Po$_2$ between 11 and 47 mm Hg. The data did not distinguish between a direct and an indirect effect of oxygen on the vascular smooth muscle of the terminal arterioles and precapillary sphincters. However, the oxygen tensions measured at these sites (18—30 mm Hg) required that the vascular smooth muscle cells respond to altered oxygen tension at levels higher than those which have been demonstrated experimentally.

KEY WORDS: blood flow arterioles precapillary sphincters diffusion limitation vasomotion vascular smooth muscle autoregulation

The partial pressures of the respiratory gases in both blood and tissue are important determinants of peripheral vascular function, and many experiments have shown that, in general, vascular resistance is directly related to the ratio of oxygen supply to oxygen demand. However, controversy exists as to whether resistance changes are mediated directly by the effects of altered oxygen tension on vascular smooth muscle cells or indirectly via altered parenchymal cell metabolism (1, 2).

Several authors have proposed that oxygen acts directly on vascular smooth muscle during regulation of blood flow (3–10), but evidence obtained by direct observation of the resistance vessels is scanty. Studies have been made on the effects of oxygen on arteriolar vasomotion and precapillary sphincter activity, but the changes in oxygen tension of the smooth muscle cells in the resistance vessels in these studies are unknown, and furthermore, the results have not been consistent. Martini and Honig (7) and Nicoll and Webb (8) have reported that increasing the oxygen tension of the inspired gas induces contraction in the microvessels. On the other hand, Roy and Brown (11) and Krogh (12) failed to...
show an effect of a change in suffusion solution Po\textsubscript{2} on the microcirculation.

An explanation for these inconsistencies might be apparent if the oxygen tension of the vascular smooth muscle cells and the tissues were known. In a previous investigation, we initiated experiments aimed at describing the relation between Po\textsubscript{2} and microvessel function (13). We showed that a longitudinal gradient in perivascular Po\textsubscript{2} existed and proposed that it might be significant in the regulation of blood flow.

The experiments in this paper were designed to further clarify the relation between Po\textsubscript{2} and vascular smooth muscle contraction in the microcirculation during alterations in the diffusion gradient for oxygen. The data indicate that the microvascular responses to changes in suffusion solution Po\textsubscript{2} cannot be entirely attributed to a direct action of oxygen on vascular smooth muscle.

**Methods**

Golden hamsters, weighing 95—135 g, were anesthetized with 60 mg/kg of pentobarbital, ip, supplemented with urethane as needed. The trachea and femoral vein were cannulated, and the hamsters were allowed to breathe spontaneously. The cheek pouches were everted, pinned out as flat sheets, slit longitudinally, and opened for viewing as a single-layer preparation. They were suffused at a flow rate of approximately 5 ml/min with a Tris-buffered salt solution of the following millimolar composition: NaCl 119.9, KCl 4.7, CaCl\textsubscript{2} 1.6, MgSO\textsubscript{4} 1.2, and Tris 21.0. pH was adjusted to 7.35. The temperatures of both the pouch and the hamster were maintained independently at 37.5°C.

The microcirculation was observed with a Leitz Labolux microscope with long-working-distance objectives between 3.5x and 20x. Vascular diameter was measured by two different methods. Precise internal vascular diameter measurements were made with a Whalen type of microoxygen cathodes with tip diameters between 2 and 6µ. The half-response time of the electrode and associated electronics was about 1 second. Electrodes were polarized at 0.7 v, and currents were measured with a Keithley 602 picoammeter. The electrodes were calibrated in a small reservoir in close proximity to the chamber holding the cheek pouch and with a common ground. The reservoir was filled with solution identical to that used in suffusion and bubbled with gases of known composition. Calibrations were made before and after measurements, and, typically, currents on the order of 10^{-11} amp were observed in room air. Generally, the two calibrations agreed within a few percent, provided large amounts of protein were not present in the suffusion solution as a result of bleeding in the pouch. Perivascular Po\textsubscript{2} measurements were made on the external wall of the vessels as previously described (13). Since electrodes were not buried in the tissue, both measurements and calibrations were made in the same solution. The measured perivascular oxygen tension was slightly dependent on electrode position relative to the surface of the vessel, and, therefore, all measurements reported in this paper were made at the midpoint of the vessel in the vertical plane with the electrode just indenting the vascular wall. When recording Po\textsubscript{2} from a spontaneously contracting vessel, this procedure ensured that the vessel wall did not pull away from the electrode tip. The electrode was placed as nearly perpendicular to the longitudinal axis of the vessel as possible. To minimize measurement errors as a result of branching and vessel size, frequent checks were made to see whether approximately the same Po\textsubscript{2} could be recorded on the surface of a parent vessel and immediately after the branch on the smaller vessel. These checks agreed within 1—2 mm Hg.

After surgical preparation, the hamster was placed on the microscope stage and allowed to stabilize for 1—2 hours before measurements were begun. During this time, the suffusion solution Po\textsubscript{2} was maintained at low levels of 10—15 mm Hg by bubbling the solution reservoirs with N\textsubscript{2}. The other oxygen tensions used in this study were obtained by bubbling with 5% O\textsubscript{2} or 10% O\textsubscript{2} to yield mean solution oxygen tensions of 47 and 54 mm Hg, respectively. For purposes of future discussion, the solutions equilibrated with the three gases will be referred to as low-, intermediate-, and high-Po\textsubscript{2} solutions.

A total of 17 hamsters was studied. Only a single series of gases was used on any pouch, and, in all but two cases, only one of the hamster's pouches was used. The sequence of experimental gases was randomized to ensure that prior treatment by one of the gases did not bias the results. In 6 hamsters, bubbling with either 10% or
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**TABLE 1**

<table>
<thead>
<tr>
<th>Solution (mm Hg)</th>
<th>Tissue</th>
<th>Large arteriole</th>
<th>Small arteriole</th>
<th>Terminal arteriole</th>
<th>Precapillary sphincter</th>
<th>Slope (Poir vs. order)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 ± 2</td>
<td>(19)</td>
<td>11 ± 1</td>
<td>48 ± 2</td>
<td>39 ± 2</td>
<td>30 ± 3</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>47 ± 1</td>
<td>(13)</td>
<td>12 ± 1</td>
<td>40 ± 2</td>
<td>32 ± 3</td>
<td>26 ± 2</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>(12)</td>
<td>(13)</td>
<td>(12)</td>
<td>(13)</td>
<td>(13)</td>
<td>(11)</td>
<td>(11)</td>
</tr>
<tr>
<td>84 ± 4</td>
<td>(6)</td>
<td>25 ± 5</td>
<td>44 ± 3</td>
<td>39 ± 4</td>
<td>32 ± 5</td>
<td>33 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± se. Number of observations is given in parentheses. Numbers in brackets are vessel orders. Correlation coefficients are 0.69, 0.61, and 0.42 for solution oxygen tensions of 11, 47, and 84 mm Hg, respectively, and all are significant at the 5% level of confidence.

*Regression of perivascular Po₂ against vessel order is significant at the 5% level.

5% O₂ was both preceded and followed by nitrogen. In these cases, the nitrogen values were averaged.

A segment of the microcirculation was chosen which included four different types of vessels based on their branching order: large arterioles, small arterioles, terminal arterioles, and precapillary sphincters. These vessel types were assigned the numerical designations of 2 through 5 from large arteriole down to precapillary sphincter. The numbers correspond approximately to the "orders" assigned the vessels of the precapillary vascular tree by Wiedeman with the addition of 5 for the sphincters (15). Typical values for internal vascular diameter were 30–50μ, 10–20μ, 7–12μ, and 4–6μ for large arterioles, small arterioles, terminal arterioles, and precapillary sphincters, respectively.

**FIGURE 1**

Changes in perivascular Po₂. Bars with hatching represent the paired differences in perivascular Po₂ between measurements obtained in low- and intermediate-Po₂ solutions. The open bars represent the differences between low- and high-Po₂ solutions. Values are shown as means ± se. Numbers in the bars are the number of experiments performed. Asterisks indicate that a significant difference was detected with Student's t-test at the 5% level of confidence.

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Two to four tissue sites were also selected. These sites were chosen to represent the least-oxygenated portions of the pouch, and they were in a region without capillaries in close proximity. The distance between a tissue site and a capillary was usually on the order of $30-60\mu$. The measurements at these sites were averaged in the presentation of data in Table 1.

**Results**

**Effects of Gas Composition on Perivascular Oxygen Tension**

Perivascular oxygen tensions are shown in Table 1, and a paired-sample analysis of the data is shown in Figure 1. The table gives mean oxygen tensions for each of the three solutions used. Also, in the last column, a semiquantitative statistical analysis of the longitudinal gradient, which used the ordinal numbers assigned to the vessels, is presented. A regression of $P_O_2$ against the ordinal number for the vessel types was done using standard least-mean-squares line-fitting techniques. This procedure showed significant regressions of oxygen tension against vessel type in all three solutions. In other words, in each solution tested, there was a statistically demonstrable longitudinal gradient in perivascular oxygen tension, thus confirming our previous findings (13). The magnitude of the gradient was dependent on the suffusion solution $P_O_2$; elevation of solution $P_O_2$ produced a decrease in the longitudinal gradient. The difference between the slopes in the low-$P_O_2$ solution and the intermediate-$P_O_2$ solution was not significant at the 5% level.

There was a significant difference between observations made in the low-$P_O_2$ solution and those made in the high-$P_O_2$ solution. The oxygen tensions of both the precapillary sphincter and the tissue remained quite constant when solution $P_O_2$ was elevated to 47 mm Hg (intermediate $P_O_2$). However, further elevation to 84 mm Hg resulted in substantial increases in both parameters.

Further analysis of these data is shown in Figure 1. This figure depicts the paired differences in perivascular oxygen tension produced by increasing suffusion solution $P_O_2$ from a mean of 11 mm Hg to either 47 or 84 mm Hg. The data clearly show a decrease in perivascular $P_O_2$ resulting from an elevation of solution $P_O_2$ to 47 mm Hg. The decreases are statistically significant at the 5% level for all but the precapillary sphincter. These data are in sharp contrast to those obtained during suffusion with the solution with a higher $P_O_2$. When solution $P_O_2$ was elevated to 84 mm Hg, perivascular $P_O_2$ was elevated compared with that measured when the solution $P_O_2$ averaged 11 mm Hg.

**Effects of Gas Composition on Vessel Diameter**

Table 2 shows the effect of alterations in suffusion solution gas composition on vessel diameter. Because of the wide range of vessel sizes observed, the data have been normalized by calculating the percent of diameter in the low-$P_O_2$ solution. A significant effect on vessel diameter could be shown for the large arterioles exposed to a solution $P_O_2$ of 47 mm Hg and for the terminal arterioles exposed to a solution $P_O_2$ of 84 mm Hg. Table 2 also shows the combined data for all vessels.

### Table 2

<table>
<thead>
<tr>
<th>Solution $P_O_2$ (mm Hg)</th>
<th>All vessels (%)</th>
<th>Large arterioles</th>
<th>Small arterioles</th>
<th>Terminal arterioles</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>47</td>
<td>87 ± 5*</td>
<td>76 ± 4*</td>
<td>92 ± 9</td>
<td>91 ± 11</td>
</tr>
<tr>
<td></td>
<td>(33)</td>
<td>(10)</td>
<td>(12)</td>
<td>(11)</td>
</tr>
<tr>
<td>84</td>
<td>80 ± 5*</td>
<td>91 ± 8</td>
<td>85 ± 7</td>
<td>68 ± 13*</td>
</tr>
<tr>
<td></td>
<td>(22)</td>
<td>(6)</td>
<td>(8)</td>
<td>(8)</td>
</tr>
</tbody>
</table>

Values are means ± se. Number of determinations is given in parentheses.

*The value is significantly different from 100% at the 5% level of confidence.
studied. The average diameter of all vessels studied was significantly reduced during suffusion with both higher levels of Po2 tested. Furthermore, the constriction was dependent on the level of solution Po2.

**Correlation of Diameter and Perivascular Oxygen Tension**

As solution Po2 was elevated over the range from 11 to 47 mm Hg, there was a vasoconstriction in the large and small arterioles (Table 2), whereas the perivascular Po2 on the average decreased (Fig. 1). In contrast, further elevation of solution Po2 to 84 mm Hg resulted in both vasoconstriction and elevated perivascular Po2.

Spontaneous diameter changes are frequently seen in the arterioles of the cheek pouch, and they are sometimes correlated with alterations in perivascular Po2. Efforts were made to examine the phase relations between these two parameters. Figure 2 illustrates two examples of spontaneous phasic vasomotion, as recorded with the video densitometer, and the corresponding changes in perivascular Po2. In neither case was there a clear-cut phase difference between Po2 and diameter, and neither record suggests that the Po2 of the vascular smooth muscle was producing the cyclic vasomotion. In no case have vessels been observed in which changes in Po2 precede changes in diameter, as would be the case if the oscillations were induced by changes in oxygen tension.

**Discussion**

For oxygen to be directly involved in the regulation of vascular function, two criteria must be satisfied. First, the local oxygen tension of the vascular smooth muscle cells must change in the appropriate direction as the relation between oxygen supply and tissue demand is altered. Second, any changes in Po2 which do occur must be within a range which
can alter smooth muscle cell contractile activity. The data shown in Figure 1 and Table 1 have a direct bearing on the first criterion.

In a previous publication, we demonstrated that the perivascular $P_{O_2}$ was very closely related to the intravascular $P_{O_2}$ (13). Thus, perivascular $P_{O_2}$ provides an estimate of the oxygen tension in the environment of the vascular smooth muscle cells. It follows then that, if oxygen is acting directly on the smooth muscle cells, elevation of the solution $P_{O_2}$ should increase the perivascular $P_{O_2}$; if, however, regulation is very precise, there might be no change in perivascular $P_{O_2}$. As shown in Table 1 and Figure 1, the perivascular $P_{O_2}$ on the large and small arterioles was actually reduced as the suffusion solution $P_{O_2}$ was elevated from 11 to 47 mm Hg.

If the measurements reported in this paper are combined with data from a previous publication (13), a more complete picture of the relation between perivascular $P_{O_2}$ and suffusion solution $P_{O_2}$ can be obtained. In Figure 3, the solution $P_{O_2}$ is plotted as the independent variable and perivascular $P_{O_2}$ as the dependent variable. Points at solution oxygen tensions of 39, 79, and 149 mm Hg come from the previous study. When the data are integrated in this form, a pattern which holds for each vessel type except the precapillary sphincter can be seen. As the solution $P_{O_2}$ is increased from the low value of 11 mm Hg, the arteriolar oxygen tension decreases and passes through a minimum. Therefore, over a range of solution $P_{O_2}$ from 11 to approximately 80 mm Hg, oxygen cannot be directly active in the control of these microvessels, because the $P_{O_2}$ decreases as the vascular diameter decreases. It should be noted that this range of solution oxygen tensions is particularly relevant to the problem of flow regulation, since it almost certainly includes oxygen tensions comparable to those found in the tissue (10).

The reduction in perivascular $P_{O_2}$ induced by an increase in suffusion solution $P_{O_2}$ can be explained by the fact that both the diffusion gradient and the blood velocity determine the $P_{O_2}$ at any point on the arteriolar network. As solution $P_{O_2}$ is increased, the diffusion gradient for oxygen from vessels to solution is reduced, but the supply of oxygen by diffusion from solution to tissues is increased. The increased diffusion of oxygen from solution to tissues results in a constriction of arterioles.
and precapillary sphincters. The constriction might either result from a direct action of oxygen at sites other than the large and small arterioles or be the indirect effect of altered tissue metabolism. In either case, the active constriction, coupled with associated passive diameter changes which might occur at other sites, would increase vascular resistance and decrease velocity all along the vascular bed. Perivascular $P_{O_2}$ would then tend to fall as a result of precapillary losses of oxygen. Thus, the two processes—decreased diffusion gradient and decreased velocity—will tend to produce opposite effects on the precapillary $P_{O_2}$. The shape of the curves in Figure 3 can therefore be explained by assuming that the velocity effect dominates in the lower end of the $P_{O_2}$ range, whereas the diffusion-gradient effect dominates at the higher end. Whalen and Nair (10) have previously observed what might be a similar phenomenon in measurements of tissue $P_{O_2}$ in intact skeletal muscle. In their experiments, deep-tissue $P_{O_2}$ fell, in many cases, when the oxygen tension at the surface of the tissue was elevated. They proposed that this was due to a constriction of the surface vessels in response to the high solution $P_{O_2}$.

In contrast to the events occurring in the larger vessels, the oxygen tensions at the precapillary sphincter and the tissue sites remained relatively constant as solution $P_{O_2}$ was elevated up to 80 mm Hg. The relative constancy of oxygen tension at these sites strongly suggests vascular regulation of either $P_{O_2}$ or another variable closely related to $P_{O_2}$. The first of the two criteria required for the direct regulation of blood flow by oxygen is thus met in the end portion of the terminal arteriole and at the precapillary sphincter, because $P_{O_2}$ at these sites remains relatively constant as the need for vascular oxygen input is altered. Therefore, on the basis of $P_{O_2}$ measurements alone, one cannot distinguish a direct effect of oxygen from an indirect one.

To analyze the behavior of the terminal arterial vessels more completely, the oxygen sensitivity of the smooth muscle cells must be known. Table 1 indicates that the smooth muscle must be capable of responding to changes in oxygen tension over the range of 18-30 mm Hg. It has been shown that strips of large arteries and perfused vascular segments relax as the oxygen tension is lowered in this range (16-18), but these data may not be applicable to the microvessels, since large differences may exist between the $P_{O_2}$ in the external environment of isolated tissues and the $P_{O_2}$ of their centermost smooth muscle cells. If extrapolations are to be made from data on isolated tissues to the performance of the microvessels, then it must be clearly shown that the interior cells in the in vitro system are exposed to oxygen tensions compatible with those existing in the environment of the microvessels. The in vitro data presently available do not clearly demonstrate such compatibility; this statement can be clarified by a simple calculation. Diffusion limits the supply of oxygen to the interior of in vitro systems, and, if the systems are assumed to behave as flat homogeneous sheets, then the $P_{O_2}$ within the tissue can be estimated by the relation $P_{O_2} = P_{O_2,s} - M/2D(Hx - x^2)$, where $P_{O_2,s}$ is the oxygen tension in the strip at some point $x$, $P_{O_2,s}$ is the solution $P_{O_2}$ at the surface of the strip, $M$ is the metabolic rate of the tissue, $D$ is the diffusion coefficient, and $H$ is the tissue thickness (19). Simultaneous measurements of all of the relevant variables and the changes in mechanical activity produced by altered oxygen tension have not been made. However, a wide range of values are currently in the literature and these have been summarized (20, 21). If extreme values for aortic strips reported in these sources are used and a wall thickness of 0.3 mm is assumed (Duling, unpublished observations), the $P_{O_2}$ at the interior of the tissue can be calculated to be anywhere from 24 to 50 mm Hg less than the solution $P_{O_2}$ as a result of diffusion limitation of $O_2$ supply. If one assumes a value of 0.12 mm for the wall thickness of the vessels used by Carrier et al. (16), then, for their high-flow vessels, the corresponding range for the difference between solution and interior $P_{O_2}$ is 5 to 21 mm Hg.
Two experimental reports currently in the literature raise additional questions with regard to the oxygen sensitivity of the smooth muscle cells. First, Howard et al. (22) have shown that, in very small vessels with thin walls, the consumption of oxygen is independent of environmental O$_2$ down to 12 mm Hg. Therefore, if contractile activity is affected by oxygen at higher oxygen tensions, a mechanism other than oxidative phosphorylation must be involved. Second, Fay (23) has shown that even in the ductus arteriosus, which is exceptional in its oxygen sensitivity, the response to oxygen can be explained by diffusion limitation of oxygen supply. Clearly, the preceding calculations and the experiments of Howard et al. (22) and those of Fay (23) do not prove that the responses of isolated vascular smooth muscle are due to diffusion limitation of oxygen supply. Consequently, the preceding calculations and the experiments of Howard et al. (22) and those of Fay (23) do not prove that the responses of isolated vascular smooth muscle are due to diffusion limitation of oxygen supply. They do show quite well, however, that additional experimental evidence is required, particularly evidence obtained from microvessels, before the relation between oxygen and vascular smooth muscle mechanical activity is clearly established and shown to be adequate to explain the participation of oxygen in the regulation of blood flow.

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**References**


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Correction

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Microvascular Responses to Alterations in Oxygen Tension
Brian R. Duling

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