Changes in Microvascular Diameter and Oxygen Tension Induced by Carbon Dioxide

By Brian R. Duling

ABSTRACT

Microvascular diameters in the hamster cheek pouch were measured with a Vickers image-shearing eyepiece, and oxygen tension (Po2) was measured amperometrically with 2-8 μm microcathodes. Perivascular Po2 was dependent on both the type of microvessel observed and the composition of the solution bathing the tissue. During application of a solution with a mean Po2 of 17 mm Hg and a Pco2 of 0 mm Hg, a longitudinal gradient in perivascular Po2 was observed: Po2 decreased from 44 ± 2 (SE) mm Hg at the large arterioles to 18 ± 2 mm Hg at the capillary origin. Tissue Po2 was 10 ± 1 mm Hg. Suffusion of the cheek pouch with a solution with approximately the same Po2 and a Pco2 of 32 mm Hg resulted in an elevation of perivascular Po2 at all sites. Large arteriolar Po2 under these circumstances was 47 ± 2 mm Hg, capillary origin Po2 was 29 ± 3 mm Hg, and tissue Po2 was 17 ± 3 mm Hg. CO2 also produced vasodilation: the average vascular diameter increased 18 ± 7% when the solution Pco2 was increased from 0 to 32 mm Hg. The tissue showed evidence of regulation of tissue O2 supply both with and without CO2 in the suffusion solution. The effects of CO2 on the distribution of O2 were compared with the effects of other vasodilators, and it was found that the tissue Po2 was not consistently changed by the application of the vasodilators, whereas it was elevated 70% by CO2. This difference is attributed in part to the effect of CO2 on the oxyhemoglobin dissociation curve.

KEY WORDS: local control vasodilators oxyhemoglobin dissociation curve vascular smooth muscle blood flow regulation Bohr effect

Methods

ANIMALS AND SOLUTIONS

In this investigation 26 golden hamsters weighing between 80 and 130 g were used. They were initially anesthetized with sodium pentobarbital (60 mg/kg, ip), and, after cannulation of the femoral vein, urethane (50%, w/v) was administered intravenously as required to replace metabolized pentobarbital. The trachea was cannulated, and the hamsters breathed spontaneously.

Preparation of the cheek pouch was carried out as described previously (22). The pouch was everted,
spreading, pinned on a Lucite pedestal, and slit longitudinally for viewing as a single layer. The surface was suffused with a warm, Tris-buffered physiologic salt solution with a millimolar composition of NaCl 119.9, KCl 4.7, CaCl₂ 1.6, and MgSO₄ 1.2. The solution was buffered with 21 mM Tris, and the pH was adjusted to 7.35. Temperature of the pouch was maintained at 37.5°C by heating the incoming fluid, and it was measured on the lower surface of the pouch with a Yellow Springs telethermometer and thermometer. The hamster's rectal temperature was maintained at 37.5°C with incandescent heating.

Suffusion solutions were equilibrated with gases of various composition to give the desired Po₂ and carbon dioxide tension (PCO₂) in the supply reservoir. Due to the flux of gas between the solution and the atmosphere, the gas composition of the solution over the pouch was different from that in the reservoir. The differences were minimized by a rapid circulation of fluid over the pouch surface.

Solution reservoirs were equilibrated with a gas mixture containing either 0, 5, or 10% O₂. The resulting mean solution Po₂ was 18, 47, or 87 mm Hg, respectively, as determined by measurement with the O₂ microcathode. These solutions will be referred to as low-, intermediate-, and high-Po₂ solutions. The O₂ mixtures were combined with either 0% CO₂ or 5% CO₂. The filler gas was nitrogen.

PO₂ determinations were made on capillary-tube samples obtained from the solution flowing over the tissue surface. The determinations were made on a Radiometer model PHA 927B gas monitor. The mean ± SE of six determinations was 32 ± 2 mm Hg, which is comparable to the expected value of 35.1 ± 0.1 mm Hg based on the observed barometric pressure and the fractional CO₂ composition. In all solutions in which 5% CO₂ was used, the pH was adjusted to 7.35 by adding sodium bicarbonate. Changes in osmolarity were avoided by a reduction in sodium chloride.

The vasodilator effect of CO₂ was compared with that of three other dilator agents which were applied in concentrations chosen to give an initial vasodilation comparable to that seen with 5% CO₂. The agents used were eledoisin (Ciba, 0.14 μg/ml), acetylcholine chloride (Nutritional Biochemical, 5 x 10⁻⁶M), and adenosine (P-L Biochemicals, 5 x 10⁻⁶M).

MEASUREMENT OF OXYGEN TENSION

PO₂ measurements were made amperometrically using the microcathode developed by Whalen (23). These electrodes had outside tip diameters of 2–6 μm, and the active surface was recessed approximately 10 μm inside a glass sleeve; the inside tip diameters were 1–3 μm. The electrodes were polarized with 0.7 V of direct current, and the resulting currents were measured with a Keithley 602 picoammeter. The response time of the electrode and its associated electronics was approximately 1 second to the half maximum response.

The electrodes were calibrated in a 50-ml heated reservoir adjacent to the pouch suffusion apparatus. A common ground wire connected the two silver–silver chloride reference wires in the calibration chamber and the pouch chamber. The calibration chamber was filled with solutions identical to those used on the pouch, and the solution was bubbled with gases of known composition. Calibration gases always contained 5% CO₂ if this gas was to be used in the suffusion solution. In all cases calibrations were made before and after measurements. Currents were on the order of 10⁻¹⁵ amp in air and decreased to less than 5% of this value in nitrogen. Perivascular PO₂ measurements were made on the external wall of the microvessels as previously described (1, 2). The electrodes were placed on the vessel wall at the midpoint in the vertical plane with the tip indenting the surface.

MEASUREMENT OF DIAMETER

The microcirculation was observed with a Leitz Labolux microscope, using long working distance objectives between 3.5x and 32x. Reference 35-mm photographs were taken with a Leitz Orthomat camera. Panatonic X film was used, and the contrast between blood and tissue was enhanced with a 540-nm interference filter in the light path.

Diameter measurements were made by two different methods. A Vickers image-shearing eyepiece was used when precise internal diameter measurements were desired. This device was coupled to a ten-turn potentiometer, and the voltage drop on the potentiometer was recorded on a Beckman Dynograph recorder. The reproducibility of this system was ±1 μm, using a 32x objective lens.

A television densitometer system coupled to the microscope was used to measure phasic changes in diameter. A photore sistive cell was mounted on the television monitor screen over the vessel to be studied, and a second reference cell was mounted over a portion of the screen which was free of vessels. The difference in resistance between the two cells was detected using a Waters XP-302 densitometer, and a voltage proportional to the resistance difference was the output for recording purposes. This system was adequate for measuring the relative diameter of the vessels; it was not suitable for absolute diameter measurements since changes in average light level and tissue position also influenced its output.

EXPERIMENTAL PROTOCOL

After surgery, the hamster was placed on the microscope stage and allowed to stabilize for 1–2 hours. During this time the suffusion solution PO₂ was maintained at low levels of 5–15 mm Hg. This procedure improves the performance and the stability of this preparation (22).

At the end of the equilibration period a segment of the microcirculation was chosen which included four different vessel types based on their position in the precapillary vasculature. The vessel types and the typical values for their inside diameters were: large arteriole (30-60 μm), small arteriole (10-20 μm), terminal arteriole (7-12 μm), and a capillary origin (4-6 μm). The capillary origin is presumably the site of the precapillary sphincter.

In addition to the vascular sites, two to four tissue sites were selected. These sites were without visible
capillaries and were chosen to represent the least oxygenated portions of the pouch. The distance between the closest capillary and a tissue site was typically 30–60 μ. Data obtained from the tissue sites were averaged for any given experimental measurement.

Twenty-six hamsters were studied during exposure of the pouch to various gas compositions including 5% CO₂. Data from 15 of these hamsters were obtained for use in a parallel investigation on the microvascular effects of O₂ alone, and these data have been published (2).

After the initial equilibration period of 1–2 hours, the gas mixture supplying the reservoir was changed, and a period of at least 20 minutes was allowed to elapse before experimental measurements were made. The sequence of gases used was randomized to ensure that observed changes were not artifactual.

Results

Perivascular Oxygen Tension

Table 1 shows the mean values of perivascular Po₂ found in this study. The last three rows give the Po₂ for each of the gas mixtures which contained CO₂. The first row gives Po₂ measured during suffusion with a low-Po₂ solution without CO₂, and this solution was used as the reference or control solution in later data analysis. The data columns are arranged to show mean Po₂ at each measurement site. With the exception of the high-Po₂ solution (Po₂ = 87 mm Hg), there was a statistically significant reduction in Po₂ along the precapillary vasculature (large arteriole to capillary origin). This longitudinal gradient of Po₂ existed both with and without CO₂ and was demonstrable with solution Po₂ levels as high as 47 mm Hg. When solution Po₂ was raised to 87 mm Hg, the longitudinal gradient was abolished and there was actually a tendency toward an increase in Po₂ along the length of the vascular tree.

The effect of an increase in CO₂ alone is seen by comparing the top two rows of Table 1. Mean Po₂ at each point in the microcirculation increased as CO₂ was increased. The Po₂ elevations observed with CO₂ were statistically significant at the 5% level for all points except the solution and the large arteriole.

The influence of solution Po₂ on perivascular Po₂ can be seen by comparing the bottom two rows with the second row in Table 1. Figure 1A illustrates this data in graphical form. Figure 1B includes data obtained previously from similar experiments done in the absence of CO₂ in the suffusion solution (2). Figure 1A shows that the longitudinal gradient was eliminated in the high-Po₂ solution. This figure and Table 1 also show that the Po₂ decreased at the small arteriole as solution Po₂ was elevated from 18 to 47 mm Hg. Such a reduction in perivascular Po₂ as solution Po₂ is elevated has been observed previously and is illustrated in Figure 1B by comparing the measurements made at a solution Po₂ of 11 mm Hg (circles) with those made at a solution Po₂ of 47 mm Hg (squares). The change from low- to intermediate-Po₂ solutions did not produce a demonstrable change in the perivascular Po₂ of the terminal arteriole, the arterial capillary, or the tissue sites in either the presence or the absence of CO₂.

Diameter Changes Induced by CO₂

Table 2 presents an analysis of the diameter changes produced by suffusion with various gas compositions. Because of the wide ranges in vessel size observed at the various vascular sites, the data were normalized by taking as 100% the vascular

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of Suffusion Solution Gas Composition on Perivascular Po₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reservoir Po₂ (mm Hg)</td>
<td>Approximate Po₂ (mm Hg)</td>
</tr>
<tr>
<td>---------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>35</td>
<td>32</td>
</tr>
<tr>
<td>70</td>
<td>32</td>
</tr>
</tbody>
</table>

Values are means ± SE. Number of observations is given in parentheses.

*Po₂ on the capillary origin is significantly different from that on the large arteriole at the 5% level.
diameter observed during application of the low-
P02 solution without CO2. As shown in Table 2, a significant increase in overall average diameter of the vessels was induced by adding CO2 to the solution, reflecting a very large effect on the large arterioles. The dilation induced in these vessels was significant at the 5% level. Table 2 also shows that the effects of CO2 were not seen if P02 was simultaneously elevated.

**TABLE 2**

<table>
<thead>
<tr>
<th>Solution composition (P02 in mm Hg)</th>
<th>Approximate Pco2 (mm Hg)</th>
<th>Large arterioles</th>
<th>Small arterioles</th>
<th>Terminal arterioles</th>
<th>All vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 ± 2</td>
<td>32</td>
<td>126 ± 8*</td>
<td>109 ± 10</td>
<td>118 ± 18</td>
<td>118 ± 7*</td>
</tr>
<tr>
<td>47 ± 3</td>
<td>32</td>
<td>96 ± 21</td>
<td>99 ± 17</td>
<td>118 ± 26</td>
<td>103 ± 12</td>
</tr>
<tr>
<td>87 ± 4</td>
<td>32</td>
<td>85 ± 15</td>
<td>108 ± 19</td>
<td>110 ± 14</td>
<td>100 ± 10</td>
</tr>
</tbody>
</table>

Number of observations is given in parentheses. Control diameter was taken as the value observed during suffusion with a solution equilibrated with 100% N2. Mean diameters in low-P02 CO2-free solution of large arterioles, small arterioles, and terminal arterioles were 48.0 ± 3.6μ, 16.0 ± 1.3μ, and 9.2 ± 1.0μ, respectively.

*Value is significantly different from 100% at the 5% confidence level.

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associated terminal arteriole which supplied the capillary is shown in the bottom trace. Eledoisin initially produced a sharp increase in Po2, but this increase was not sustained; over the next 5 minutes mean Po2 returned approximately to control levels even though exposure to eledoisin was continued.

The diameter of the terminal arteriole tended to follow the same pattern, but changes were very small. The variations in Po2 shown in Figure 2 were largely due to activity in an upstream small arteriole rather than to activity in the terminal arteriole.

Appearance of or increase in magnitude of phasic diameter changes was a common finding after vasodilator application and is illustrated in these experiments by the effects of diameter changes on Po2 during the recovery phase. During prolonged application of vasodilators, at least one of the four vessel types studied in each of the eight experiments was observed either to begin oscillating or to increase the magnitude of existing oscillations.

Discussion

As anticipated, CO2 altered both the diameter and the Po2 of microvessels. It produced an increase in perivascular Po2 at all points except the large arteriole. Also, in the presence of CO2 a longitudinal gradient in perivascular Po2 persisted, although its magnitude was diminished. These findings confirm previous observations and further demonstrate that significant quantities of O2 can be lost from the precapillary vasculature (1). As shown in Table 2, CO2 caused vasodilation in the low-Po2 solution. These data are in agreement with previous reports of a CO2-induced vasodilation in the microcirculation (16-21) and also with reports of a decrease in resistance of various vascular beds (3-14). Quantitatively, the effect of CO2 was approximately equal to that seen during topical application of CO2 to the brain by Kontos et al. (17).

At higher solution Po2 levels, it was not possible to show an effect of CO2 on vascular diameter. However, it should be recognized that the measurements made in the intermediate- and the high-Po2 solutions reflect simultaneous changes in both Po2 and Pco2. The effects of O2 alone have been reported previously: a 13% reduction in diameter was observed as solution Po2 was increased from 11 mm Hg to 47 mm Hg, and a 20% reduction was associated with an increase from 11 mm Hg to 84 mm Hg (2). The lack of effect of O2 at higher solution Po2 was probably due to the interaction between the constrictor effect of O2 and the dilator effect of CO2.

The changes in Po2 observed during application of CO2 were probably due to both the vasodilator effect of CO2 and its effect on the oxyhemoglobin dissociation curve, but the available data do not permit a quantitative separation of the two effects. By considering the shifts in the oxyhemoglobin dissociation curve associated with various changes in CO2, it can be shown, however, that the effect of CO2 could be large enough to explain the observed changes. Assuming that the oxyhemoglobin saturation remains constant at the entrance to the capillaries, the published value for the Bohr shift can be used to predict the change in CO2 required to produce the observed change in Po2. The Po2 at the capillary origin during exposure to CO2 is taken as 29 mm Hg (Table 1, line 2) and during exposure to CO2-free solution as 18 mm Hg (Table 1, line 1). It is assumed that intravascular CO2 is in equilibrium with solution CO2 during suffusion with the high-Pco2 solution (i.e., 32 mm Hg) and also that the plasma bicarbonate concentration is 21 mEq/liter at a pH of 7.4. The value for the Bohr shift requires a pH change of 0.51 units for a change in Po2 of 11 mm Hg. This pH change corresponds to reducing the Pco2 in the blood from 32 to 12 mm Hg, which is not unreasonable during exposure to the CO2-free solution. Thus, it is at least possible for alterations in intravascular CO2 to
produce changes in Po2 of the magnitude measured at the capillary origin.

There is no way of assessing the possible contribution of vasodilation per se to the net change in Po2. However, as was shown by the use of other dilators, substantial changes in Po2 can be produced by vasodilation alone. However, in view of the fact that the distribution of Po2 in the presence of CO2 was much different from that observed with other dilators and also the fact that the Po2 changes induced by dilators other than CO2 were transient, it seems likely that much of the effect of CO2 reflects a shift in the oxyhemoglobin dissociation curve.

The results of these experiments raise an interesting question with regard to the possible role of O2 in local blood flow regulation. O2 might be involved in coupling flow and metabolism by either a direct action on smooth muscle cells or a secondary effect as a result of its influence on parenchymal cell metabolism. Hypotheses have been advanced to support both possibilities (22).

It has been shown in an investigation which was carried out in parallel with this one that the Po2 at the capillary origin and in the tissue remains relatively constant over a wide range of solution Po2 (2). This finding was presented as evidence that either Po2 or some parameter closely related to it was regulated in the cheek pouch preparation. Figure 3 compares the results of that study with data from Table 1. The patterns for both the capillary origin and the tissue were quite similar qualitatively, both with and without CO2. That is, in the low- and the intermediate-Po2 solutions, perivascular and tissue Po2 changed little but then increased sharply when tissues were exposed to the high-Po2 solution.

Consideration of the quantitative relations shown in Figure 3 evinces a clear-cut difference between the data obtained with and without CO2. In each case, perivascular Po2 was elevated when CO2 was added. However, the relative constancy of Po2 in the low- and the intermediate-Po2 solutions suggests that regulation of some type persists. That is, addition of CO2 to the suffusion solution results in a condition in which the smooth muscle cells continue to respond to changes in O2 demand in the appropriate fashion, but they do so at a higher Po2.

Two possibilities might explain this finding. First, if O2 supply is regulated by a direct effect of O2 on vascular smooth muscle, then perhaps the relation between smooth muscle contraction and Po2 is shifted in the presence of CO2. To my knowledge, the necessary experimental data do not exist to evaluate this possibility. However, in view of the findings with other vasodilators (Fig. 2), the effect of CO2 would have to be relatively specific.

A second possibility is that O2 acts only through an effect on parenchymal cell metabolism. In this case, one would expect that the rate of O2 delivery to the cells per unit time would be regulated rather than the absolute quantity of O2. As long as the oxyhemoglobin dissociation curve is unaltered, such regulation would result in a constant tissue Po2 as O2 demand is altered. However, if the dissociation curve is shifted, then the tissue Po2 would change so as to give a constant rate of cellular O2 delivery. This possibility is attractive because of its simplicity: no resetting of the control system is required as PO2 is altered. A clear separation of the two possibilities might be obtained by increasing the O2 content of the blood by other mechanisms or by measuring the microvascular O2 content directly.

References


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