The Oxygen Sensitivity of Hamster Cheek Pouch Arterioles

In Vitro and in Situ Studies

William F. Jackson and Brian R. Duling

From the Department of Physiology, University of Virginia School of Medicine, Charlottesville, Virginia

SUMMARY. We tested the hypothesis that a parenchymally derived mediator is required for arterioles to exhibit oxygen sensitivity. To that end, the parenchyma was dissected and removed from around hamster cheek pouch arterioles, and the oxygen sensitivity of these "aparenchymal arteriolar segments" was studied, either in vitro, after cannulation, or in situ. Arteriolar segments in situ with and without parenchyma had similar oxygen sensitivities (20% constriction as PO2 increased from 15 to 150 mm Hg). Arteriolar occlusion, which eliminated blood flow in the in situ aparenchymal segments, did not eliminate their oxygen sensitivity. The oxygen-induced constriction in the occluded aparenchymal segments was blunted but not eliminated by covering the segments with glass plates to prevent changes in P02 from occurring around these vessels. We hypothesized that propagation of a portion of the oxygen response might explain the persistent response in the covered and occluded arteriolar segments. Oxygen sensitivity could be shown in only 32% of the in vitro cannulated arterioles (16% mean constriction as P02 increased from 20 to 150 mm Hg). In contrast, 75% of aparenchymal arterioles were sensitive to changes in P02 in situ. These data led us to reject the hypothesis that a parenchymally derived mediator is absolutely required for arterioles to exhibit oxygen sensitivity. We infer that the oxygen sensitivity of hamster cheek pouch arterioles results partially or totally from the local action of oxygen on some component of the arteriolar wall or blood, that a portion of the oxygen response may be the result of a propagated phenomenon, and that the oxygen-sensitive component is fragile and is easily lost in preparation for in vitro measurements or in cannulation. It is emphasized that the O2 sensor need not reside in vascular smooth muscle. (Circ Res 53: 515-525, 1983)

THERE is a close relationship between oxygen supply (the product of blood flow and blood oxygen content) and oxygen demand (metabolic rate) in many tissues (see Sparks, 1980). This correlation suggests that oxygen is involved in the control of microvascular function. A number of microvascular studies have shown that increased PO2 in the environment of arterioles results in arteriolar constriction (Duling, 1972, 1974; Hutchins et al., 1974; Sullivan and Johnson, 1981). It is not known whether changes in PO2 are sensed by a primary action on parenchymal cells, or by the arterioles themselves.

In most studies, it has not been possible to distinguish between a primary action of oxygen on resistance vessels or on parenchymal cells because of the close association between arterioles and tissue, and because of the interrelation of tissue and vessel PO2. As a result, most investigators have tried to evaluate the two possibilities by studying the oxygen sensitivity of vascular smooth muscle in strips, rings, or segments of relatively large conduit or elastic arteries in vitro (Carrier et al., 1964; Pittman and Duling, 1973; Coburn et al., 1979; Chang and Detar, 1980). Whereas these investigations have shown that arteries are sensitive to changes in PO2, their relevance to arteriolar oxygen sensitivity and to the role of oxygen in the local control of blood flow remains in question.

Therefore, we have reinvestigated this question using recently developed techniques that allow arterioles to be separated from the surrounding parenchyma and studied in situ or in vitro. Results from our experiments suggest that a parenchymally derived mediator is not essential for hamster cheek pouch arterioles to respond to oxygen, and that arterioles or elements in arteriolar blood may act as the site of action of oxygen in the system.

Methods

Preparation of Animals

Male golden hamsters (105 ± 1.49 g, n = 73) were anesthetized with sodium pentobarbital (Nembutal, Abbott; 70 mg/kg administered intraperitoneally). Tracheal and femoral venous cannulae were inserted and the animals received an infusion (0.44 ml/hr) of Nembutal diluted with saline (9.1 mg/ml) to maintain the appropriate level of anesthesia and to replace fluids lost due to surgery, renal function, and respiratory evaporation. Fluid infusion at this rate maintained animal hematocrits constant and mean arterial pressure constant at 100 mm Hg for up to 6 hours. Deep esophageal temperature was maintained at 37°C by conductive heating.
Preparation of Cheek Pouches

Cheek pouches were prepared for viewing as a single layer preparation by standard methods (Duling, 1973). Pouches were suffused (10 ml/min) with warm physiological salt solution (PSS) buffered with bicarbonate (composition in mm: NaCl, 145; KCl, 4.7; CaCl2, 2; MgSO4, 1.17; Na-EDTA, 0.02; glucose, 5; pyruvate, 2; NaHCO3, 20). Solutions were gassed with mixtures containing 5% CO2 and various fractions of O2 and N2. The pH of the suffusion solution was 7.4 and the temperature was 37°C. Pouches were suffused with solutions equilibrated with 95% N2 and 5% CO2 during preparation of the cheek pouch and subsequent dissection of individual arterioles. The PO2 of the solution flowing over the pouch, measured with oxygen microelectrodes (Whalen et al., 1967), was approximately 15 mm Hg.

Second and third order arterioles (in vivo diameter range = 20-60 μm) from the epithelial portion of the cheek pouch were chosen for study. These arterioles lie superficially on the epithelium in loose areolar connective tissue. Using microdissection techniques (Duling et al., 1981), a long segment of arteriole was freed from the surrounding connective tissue and the underlying parenchyma. A circular piece of parenchyma approximately 2 mm in diameter was then cut out and removed, leaving a long, autoperfused arteriolar segment spanning a parenchyma free hole in the cheek pouch [i.e., an aparenchymal arteriolar segment (see Fig. 1)].

Preparation and Cannulation of Arterioles for Study in Vitro

For in vitro studies, arterioles were selected with unbranched segment lengths of 0.5-1.0 mm and were freed from the parenchyma and connective tissue as outlined above. The suffusate flow then was stopped and the ends of the vessel segment were cut with very sharp scissors. The arteriolar segment was then transferred to a 3-ml chamber mounted on the stage of an inverted microscope for cannulation and study (Duling et al., 1981). The vessel chamber was initially filled with PSS buffered with morpholinopropanesulphonic acid (MOPS, Sigma) containing 1% bovine serum albumin (dialyzed fraction V, Sigma) (in mm: NaCl, 145; KCl, 4.7; CaCl2, 2; MgSO4, 1.2; glucose, 5; pyruvate, 2; Na-EDTA, 0.02; MOPS, 2). This MOPS-buffered PSS had previously been equilibrated with 100% N2 and had a measured PO2 of between 10 and 30 mm Hg.

One end of the arteriole was cannulated and the other end sealed as described by Duling et al. (1981). The vessel then was pressurized to 40 mm Hg with MOPS-buffered PSS. We chose 40 mm Hg inflation pressure because it represented a pressure between the 30 mm Hg measured in 20-μm arterioles in the epithelial portion of cheek pouches (Lombard and Duling, 1977a) and the 55 mm Hg measured in 50-μm arterioles in the more proximal muscular portion of cheek pouches (Davis et al., 1981). Also, the in situ pressure in 30 to 40-μm arterioles in the epithelial portion of cheek pouches has recently been measured to be approximately 40 mm Hg (Michael J. Davis, personal communication). Some blood was allowed to remain in cannulated vessels so that the red cells could be used as flow markers to detect any perfusate leakage from the cannulated arterioles. The cannulation procedure was carried out at room temperature (19-21°C). The vessel chamber was then perfused (2-5 ml/min) with 37°C bicarbonate-buffered PSS equilibrated with 95% N2, 5% CO2, and the cannulated arterioles were allowed to stabilize for 15-30 minutes.

Cannulated arterioles were visualized with a videomicroscopy system at a final magnification of 2000× measured at the face of the monitor. Vessel inside diameters were measured with a videomicrometer accurate to ±1 μm.

Reactivities of Arterioles in Vitro

Oxygen sensitivity was assessed by changing the gas with which the vessel chamber perfusate was equilibrated, from gas containing 0% O2 to gas containing 21% O2 and then back to the 0% O2 gas. These two oxygen levels represented PO2 values of approximately 20 and 150 mm Hg, respectively, measured in the vessel chamber with oxygen microelectrodes. This procedure was repeated 2-5 times for each vessel and the diameter of the vessel was measured under each condition.

For comparative purposes, we also tested the norepinephrine (Levophed bitartrate, Sterling) sensitivity of the cannulated arterioles by placing 25-100 μl of a 10 μM to 1 mM norepinephrine solution into the 25-ml perfusate reservoir. Concentrations of norepinephrine given in the text are the maximum concentration of drug delivered to the vessels based on a 1000- to 250-fold dilution.

Preparation of Arterioles for Study in Situ

Long (1-3 mm) arterioles were selected for study in situ, and the parenchyma was removed from beneath the vessels as described above. Undissected portions of the arterioles, approximately 2 mm upstream or downstream from the parenchyma-free arteriolar segments, were termed "control segments," and were used as a control for the effects of surgical trauma and to assess the effects of the parenchyma on the response of arterioles.

Preparation of Covered Arterioles

To prevent PO2 changes made in the suffusion solution from reaching the aparenchymal segments, we covered the segments and the hole in the cheek pouch with small...
rectangular plates of glass, cut from no. 1 coverslips, 0.5–1 mm larger than the hole in the pouch parenchyma. The glass plates were tightly sealed in place with silicone stopcock grease (Dow). The grease was placed under the segment and filled the hole around the aparenchymal segment. In addition, a bead of the grease was placed along the surface of the cut edge of the pouch. The glass plate then was carefully pressed into the grease over the aparenchymal segment so that any excess grease was pressed away from the edges of the glass plate. As a control for the effects of the silicone grease on the vessels, most experiments were conducted with the grease around the vessel, but with no cover over the vessel.

Preparation of Occluded Arterioles
To eliminate blood flow and, hence, convection of blood and any dissolved substances into parenchyma-free arteriolar segments, vessels were occluded randomly, either upstream or downstream from the dissected segments, with heat-bubbled glass micropipettes mounted on a micromanipulator. Uncovered, as well as covered, parenchyma-free vessels were studied in the occluded state.

Measurement of Diameter and Hemoglobin Saturation in Situ
In most experiments, transilluminated arterioles were visualized through a microscope fitted with a Leitz UMK 32-long working-distance objective and 20× eyepieces. Vessel inside diameters were measured with an eyepiece micrometer. In experiments where the PO2 sensitivity of vessels was determined over the range of 0%–95% oxygen content of the superfusate, vessels were visualized with a videomicroscopy system at 2000× measured at the face of the monitor, and vessel inside diameters were measured with a videomicrometer accurate to ±1 μm.

Arteriolar blood hemoglobin oxygen saturation was measured by a microspectrophotometric technique (Pitman and Duling, 1975; Duling et al., 1983). This method consists of simultaneous determination of the absorbance of blood at three wavelengths: two isosbestic wavelengths for correction due to light scattering, and one wavelength sensitive to the relative amounts of oxy- and deoxyhemoglobin.

Reactivities of Arterioles in S itu
We examined the oxygen sensitivity of aparenchymal arterioles by measuring the diameters of the segments with 0%, 10%, 21%, and 95% O2 in the superfuse. These conditions yielded sulfusate PO2 values of approximately 15, 80, 150, and 500 mm Hg, respectively, measured in the fluid flowing over the pouch.

In tests of the efficacy of various experimental manipulations in altering the oxygen response of aparenchymal segments, oxygen sensitivity was assessed by varying the gas that was bubbled through the superfusion solution from one containing 0% oxygen (PO2 approximately 15 mm Hg) to one containing 21% oxygen (PO2 approximately 150 mm Hg), and then back to the 0%–O2-containing gas. At least two such tests were performed under each experimental condition, and the diameter measurements made during the trials were averaged.

Reactivities to adenosine and norepinephrine were assessed by topical application of 10–25 μl of 100 μM adenosine (Sigma) or 10–15 μl of 0.29 and 2.9 μM norepinephrine (Levophed, Sterling) into the superfusion solution flowing over the cheek pouch. Solutions of adenosine and norepinephrine were prepared fresh each day in PSS and stored on ice while not in use.

Lidocaine Experiments
We assessed the effects of the local anesthetic lidocaine on the oxygen sensitivity and norepinephrine reactivity of parenchyma-free and control arteriolar segments by quantifying their oxygen responses and norepinephrine reactivities before and after pouches were exposed to 1 mM lidocaine. Cheek pouches were exposed to lidocaine for 30–60 minutes prior to assessment of oxygen sensitivities.

Lidocaine (lidocaine base, Sigma) was dissolved in 1 n HCl to yield a concentration of 0.5 M. An aliquot of this concentrated solution then was added to PSS to yield a final concentration of 1 mM, and the pH was adjusted to 7.4 with NaOH.

Statistics
Treatments were assigned randomly in all experiments, and data are expressed as means ± sE. Blocked analyses of variance and the Student’s t-test on paired Student’s t-tests were used to assess treatment effects and compare mean values (Sokal and Rohlf, 1969). All statistical comparisons were performed at the P < 0.05 level.

Hypotheses to be Tested
To determine whether the parenchyma is required for arterioles to exhibit oxygen sensitivity, we tested three hypotheses, based on the idea that oxygen could elicit an arteriolar response by an initial effect on parenchymal cells resulting in a change in the rate of production of a vasoactive metabolite. Given this model, there are three cogent hypotheses which might explain the actions of a parenchymally derived mediator. Hypothesis 1: the metabolite might act locally, within a limited distance of its site of production to produce an arteriolar response. Hypothesis 2: the metabolite could diffuse into the arteriolar blood and be convected to distant sites to exert its effect. Hypothesis 3: a local arteriolar response, initiated by a parenchymally derived mediator, could be propagated along the length of an arteriole to regions distant from the site of production of the mediator. Propagation could be mechanical in nature (i.e., initiated by changes in intravascular pressure due to vasomotor activity upstream or downstream from the segment of arteriole in question), or it could be a cellularly conducted phenomenon. We include the latter mechanism because arterioles in the cheek pouch demonstrate propagated vasodilation (Duling and Berne, 1970a), and because periarteriolar nerves, vascular smooth muscle (Johansson and Somlyo, 1980), and endothelial cells (Northover, 1980; Larson and Sheridan, 1982) are electrically active and possibly coupled.

In our tests of these hypotheses we assumed that (1) the dissection procedure effectively removed parenchymal cells from around arterioles, (2) convective transport of any vasoactive substance (other than oxygen) in effective concentrations in the superfusion solution is unlikely because of dilution due to the high flow of the superfusion solution, and (3) covering and occluding an aparenchymal arteriolar segment prevents PO2 changes in the environment of the covered occluded segment.

Based on the three assumptions above we can, a priori, predict the outcome of experiments in relation to the three hypotheses (see Table 1). If the oxygen sensitivity of the cheek pouch arterioles depends solely on the local action of a parenchymally derived mediator (first row, Table 1),
and labile, + = responds to Po2 change, but magnitude of the response is greatly diminished. See text for more information.

then only arteriolar segments with intact parenchyma (CS in Table 1) should respond when suffusion solution Po2 is changed. Similarly, if convection of a parenchymally derived mediator is responsible for the oxygen sensitivity of arterioles, then only control segments, parenchymal segments, and covered parenchymal segments should be oxygen sensitive (second row Table 1). Finally, if the effect of an oxygen-related, parenchymally derived mediator can be propagated along the arteriole, then all preparations except the isolated cannulated vessels should be oxygen sensitive (third row, Table 1).

Results

Oxygen Sensitivity of Aparenchymal Arterioles in Vitro

Of the 28 arterioles studied in vitro, only nine responded in a consistent reproducible fashion when vessel chamber Po2 was varied. The other 19 arterioles displayed no repeatable oxygen response. In the nine arterioles, increasing vessel chamber Po2 from approximately 20 to 150 mm Hg, resulted in a significant decrease in internal diameter from 51.6 ± 3.8 to 43.2 ± 3.2 μm (paired difference = 8.4 ± 1.6 μm; significantly greater than zero, P < 0.05). The oxygen reactivity in these nine vessels could not be maintained. Rather, after 2–6 responses (mean = 4) were recorded, the oxygen sensitivity would vanish.

The failure to respond to oxygen is not an indication of general damage to the vessels. Isolated cannulated arterioles develop tone (i.e., reduce diameter at constant pressure) when vessel chamber temperature is raised from room temperature to 37°C (Duling et al., 1981). Both classes of arterioles in the present study developed similar levels of spontaneous tone. The oxygen-sensitive vessels constricted by 11 ± 3 μm (mean diameter at room temperature = 63 μm) when warmed to body temperature, whereas the arterioles that were oxygen insensitive constricted by 9.6 ± 3 μm (mean diameter at room temperature = 53 μm) when vessel chamber temperature was elevated to 37°C. The same level of spontaneous tone was retained in the oxygen-sensitive vessels after they lost their ability to react to oxygen.

The low success rate, high variability, and lability of the vascular response to oxygen that we observed in vitro could have resulted from trauma incurred during isolation and cannulation or from removal of some critical component (physical or biochemical) required for oxygen sensitivity. There did not appear to be any correlation between the amount of adventitia (and, hence, possible parenchymal cells) left on the cannulated arterioles and the oxygen responsiveness of the vessels. Several vessels that responded to altered Po2 had very little periarteriolar connective tissue and no apparent cellular material on their surfaces. Thus, the data do not appear to be explained by the chance presence of parenchymal cells included with the arterioles.

As a possible control for trauma, we compared, where possible, the response of arterioles with and without oxygen sensitivity to a submaximal challenge with norepinephrine (59 nM). We found that the norepinephrine sensitivity of the two groups of vessels was similar (43.1 ± 14% constriction for the O2-sensitive arterioles vs. 27.6 ± 10% constriction for the O2-insensitive vessels, n = 7 for both groups).

Also, the norepinephrine reactivity in the nine oxygen-sensitive vessels did not change after they lost their reactivity to oxygen.

We also tested to see whether the addition of 0.1% albumin to the suffusate used during dissection and heparinization (1000 IU/100 g body weight Na-heparin, iv) of the animal prior to removal of arterioles from the cheek pouch would increase the occurrence of oxygen sensitivity in the isolated cannulated vessels. These measures did not appear to increase the success rate or the magnitude of the oxygen response in these vessels.

Oxygen Sensitivity of Aparenchymal Arterioles in Situ

In contrast to our findings in vitro, 75% of the parenchyma-free arterioles studied in situ responded when suffusate oxygen tension was elevated and responsiveness could be maintained for more than 4 hours. Increased suffusate oxygen content from 0% to 10%, 21%, and 95% O2 resulted in a dose-dependent decrease in arteriolar diameter and a dose-dependent increase in arteriolar blood oxygen saturation in the aparenchymal arterioles (Fig. 2).

Comparison of the Reactivities of Aparenchymal Arterioles in Situ and in Vitro

Because we obtained such consistent results in situ, we performed an additional set of experiments comparing the oxygen, norepinephrine, and adenosine reactivities of aparenchymal arterioles in situ with the same arterioles in vitro after cannulation. The results of these experiments are shown in Figure 3. The diameters of aparenchymal arterioles in vitro

<table>
<thead>
<tr>
<th>Hypotheses</th>
<th>IS</th>
<th>CS</th>
<th>AS</th>
<th>OAS</th>
<th>CAS</th>
<th>COAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Local</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2. Convected</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3. Propagated</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Results</td>
<td>+?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

IS = isolated cannulated arterioles, CS = control arteriolar segments, AS = aparenchymal arteriolar segments, OAS = occluded aparenchymal segments, CAS = covered aparenchymal segments, COAS = covered and occluded aparenchymal segments. +? = responds to Po2 change, - = no response to Po2 change, + = responds to Po2 change, but magnitude of the response is variable and labile, + = responds to Po2 change, but magnitude of the response is greatly diminished. See text for more information.

Circulation Research/Vol. 53, No. 4, October 1983
Oxygen response of aparenchymal arterioles in situ. The oxygen content of the suffusion solution is shown on the abscissa, with mean arteriolar diameter (panel A) or hemoglobin % saturation (panel B) on the ordinate. Data are expressed as means ± se with the number of observations shown above the error bars. An analysis of variance and subsequent comparison of mean values yielded the following results (means underscored by the same line are not significantly different, P < 0.05):

<table>
<thead>
<tr>
<th>%O2</th>
<th>Diameter (μm)</th>
<th>% Saturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>38</td>
<td>64</td>
</tr>
<tr>
<td>10</td>
<td>31</td>
<td>73</td>
</tr>
<tr>
<td>21</td>
<td>28</td>
<td>84</td>
</tr>
<tr>
<td>95</td>
<td>24</td>
<td>92</td>
</tr>
</tbody>
</table>

were always greater than the diameters of the same vessels in situ. All six arterioles responded to elevated P02 in situ, whereas only three of the six vessels were oxygen sensitive after cannulation in vitro (data in Fig. 3 include nonresponders). Also, in situ, all six arterioles constricted by approximately 61% when treated with 59 nM norepinephrine. In vitro, five of the six arterioles exhibited norepinephrine reactivity identical to that seen in situ (53% constriction including one nonresponder). Adenosine (10 μM) diluted all arterioles in situ by approximately 17 μm, whereas only one of the cannulated arterioles, which was also oxygen sensitive, dilated in response to adenosine. The cannulated arterioles did have some tone, however, and would dilate when exposed to the cholinomimetic methacholine (0.2–20 μM). The mean maximum diameter of the arterioles in vitro was 61 ± 5 μm when the vessels were exposed to 20 μM methacholine. Thus, in spite of similar norepinephrine sensitivity in vitro and in situ, the oxygen and adenosine reactivities of the arterioles were reduced in vitro, compared with the same arterioles in situ.

To determine whether simply cutting the arterioles eliminated their oxygen reactivity, we severed one end of four aparenchymal arterioles in situ, (two upstream and two downstream), held the severed end of the arterioles with glass micropipettes, and tested their oxygen sensitivity. All four arterioles tested exhibited oxygen reactivity, the magnitude of the responses being comparable to those observed in occluded arterioles (see below), and there was no apparent difference in the response of vessels severed upstream or downstream from the site of observation. Thus, the trauma induced by simply transecting the arterioles cannot explain the results obtained in cannulated arterioles in vitro.

Oxygen Sensitivity of Arterioles with and without Parenchyma Present

Undissected portions of arterioles upstream and downstream from parenchyma-free segments, and their aparenchymal segments, responded identically to elevation of suffusion solution P02 (Fig. 4). Also, there were no differences between the diameters of vessels in 0% O2 before or after exposure to 21% oxygen (Fig. 4). Thus, we pooled all diameter data collected under the 0% O2 conditions within each group in all subsequent analyses. A blocked analysis of variance and subsequent comparison of mean diameters indicated that there was no significant difference between the O2 response of aparenchymal arterioles and control segments. There was a slight increase in the diameters of the aparenchymal arterioles, compared with the control segments, but the difference was not significant.

Figure 4 also demonstrates that the parenchyma-free segments and control segments responded sim-
Oxygen Sensitivity of Aparenchymal Arterioles

Aparenchymal arteriolar segments were occluded to prevent vasoactive material produced by the parenchyma from entering the blood and being transported to the arteriolar segment. Elevation of the suffusate $P_{O_2}$ produced a vasoconstriction in the occluded vessels whose magnitude was greater than that observed in the unoccluded state (Fig. 5). There was no difference between the response of vessels occluded upstream and downstream from the aparenchymal segment. Thus, blood transport of some mediator is not required for arterioles to respond to changes in $P_{O_2}$.

The increased oxygen sensitivity observed in occluded aparenchymal arterioles (Fig. 5) is probably the result of a greater change in $P_{O_2}$ seen by occluded arterioles when suffusate oxygen content was elevated from 0% to 21%. Hemoglobin oxygen saturation rose from 45% to 99% when the vessel was occluded and from 59% to 69% when blood was flowing in the vessel. These data are consistent with our hypothesis and the results of Lombard and Duling (1977b), who found that when suffusion solution $O_2$ content was elevated from 0% to 10%, periarteriolar $P_{O_2}$, measured with oxygen microelectrodes, increased more in occluded arterioles than in the same vessels with blood flowing in their lumens.

Oxygen Sensitivity of Covered and Occluded Aparenchymal Arterioles

A parenchymally derived mediator could still be responsible for the observed arteriolar oxygen response if the initial effect of the mediator on a control segment could be propagated. To test this hypothesis, we covered aparenchymal segments with glass plates to prevent direct access of the oxygen in the suffusate to the surface of the vessel. Simply covering the segments did not affect their $O_2$ response (Fig. 6). The covered segments were then occluded to eliminate blood oxygen transport and the oxygen response of these covered occluded aparenchymal arterioles examined. The magnitude of the arteriolar constriction induced by oxygen was significantly attenuated when the covered segments were occluded, but the covered-occluded parenchyma-free arterioles still exhibited a significant constriction when suffusate $P_{O_2}$ was elevated (Fig. 6).

The diminution of the oxygen response in the covered and occluded segments is not the result of a reduction in the general reactivity of the vessels.
due to the covering and occluding procedure. Simply covering or occluding vessels did not decrease their oxygen reactivity, and arterioles that had been covered and occluded had normal oxygen reactivity after the cover was removed or the occlusion was released.

As noted above, simply covering the segments did not affect their oxygen sensitivity. This could have resulted from either propagation of the response along the vessel or continued convection of blood and oxygen into the covered segment. In one experiment, we measured the hemoglobin oxygen saturation in a segment before and after it was covered. When suffusate oxygen content was elevated from 0% to 21%, arteriolar blood oxygen saturation increased from 59% to 69% in the uncovered segment and from 65% to 69% when the vessel was covered with glass. Thus, there was oxygen transport under the cover when blood was flowing in the lumen of the arteriole.

The hemoglobin oxygen saturation measured in a covered occluded segment did not change when suffusate oxygen content was elevated from 0% to 21%. Thus, we could prevent changes in Po2 from occurring in the parenchyma-free segments by covering and occluding the vessels.

As a further test of the efficacy of the covering and occluding procedure in preventing the changes in suffusate or blood oxygen tension from affecting the segments, we applied 2.9 μM norepinephrine topically. A perfect seal would have been indicated by the complete absence of a response underneath the cover. It was not possible to completely eliminate the norepinephrine-induced constriction in all experiments (14.9 ± 6.1% constriction, n = 14). However, there was no significant correlation between the change in diameter produced by norepinephrine (D-NEPI, μm) and the observed O2 constriction (D-02, μm) (D-O2 = 0.12 × D-NEPI + 1.29 μm, r = 0.4164, n = 14) in covered and occluded vessels, suggesting that simple leakage of oxygen under the cover cannot explain our results.

**Lidocaine Experiments**

The results of the experiments involving covered and occluded segments, summarized above, could be explained if a portion of the arteriolar O2 response was propagated along the length of the arteriole. As a further test of this hypothesis, we assessed the effects of the local anesthetic lidocaine on the oxygen sensitivity of the parenchyma-free arterioles. We chose lidocaine because this drug has been shown to block propagated vasodilation in cheek pouch arterioles (Duling and Berne, 1970a). Lidocaine (1 mM) produced a sustained vasoconstriction in control and parenchymal segments (Figs. 7 and 8), eliminated the O2 sensitivity in both types of segments, and greatly reduced the norepinephrine reactivity in the vessels (Fig. 8). Thus, the effects of lidocaine appear to be nonspecific. We also looked at the O2 response of the vessels exposed to 10 μM 0.1 mM, and 10 mM lidocaine and found that, except for the 10 μM dose (which was without effect), the results were comparable. The constrictor activity of lidocaine we observed confirms the observations of Duling and Berne (1970a) and others (see Bolton, 1979).
Discussion

Our results and the hypotheses we tested are summarized in Table 1. Isolated cannulated arterioles were sensitive to changes in \( P_{O_2} \) but the response was labile and could not be elicited in every experiment. Arteriolar segments with and without surrounding parenchyma (control segments and aparenchymal arteriolar segments, respectively) had similar oxygen sensitivities. Eliminating blood flow in the lumens of the aparenchymal arteriolar segments did not eliminate their \( O_2 \) sensitivity. Elimination of blood flow and contact with the suffusion solution (i.e., by covering and occluding the aparenchymal arteriolar segments) diminished but did not completely eliminate the \( O_2 \) response. In the discussion to follow, we examine the relevance of these findings to the various hypotheses advanced to explain the oxygen sensitivity of cheek pouch arterioles.

Based on our oxygen sensitivity studies on in vitro cannulated arterioles, and the oxygen sensitivity of aparenchymal arteriolar segments in situ, we can reject the hypothesis concerning a local action of a parenchymally derived mediator whose production is \( P_{O_2} \) dependent. We draw this conclusion because no parenchymal cells were associated with the arterioles in either case. This conclusion depends heavily on two assumptions: (1) that parenchymal cells were completely removed from around the aparenchymal arterioles in vitro and in situ and (2) that vasoactive substances produced by the parenchyma could not be carried to aparenchymal segments in vasoactive amounts. We feel assumption 1 is valid because the arterioles used in the present study lie in superficial connective tissue of the pouch epithelium and are not embedded in metabolically active tissue. Therefore, after dissection, an insignificant number of cells could have remained on the surface of the vessels.

The second assumption, that a parenchymally derived mediator cannot be carried in vasoactive amounts in the suffusate, also appears reasonable. The suffusate flow rate (10 ml/min) was high relative to the volume of tissue it suffused (0.1 g wet weight) and, therefore, any parenchymally derived mediator should have been greatly diluted. Furthermore, if the oxygen response of aparenchymal arterioles in situ resulted from suffusate transport of a parenchymally derived mediator, the oxygen response of aparenchymal arterioles should have been much less than the response of control arterioles with intact parenchyma due to dilution of the metabolite. The oxygen response of aparenchymal arterioles was identical to arterioles with intact parenchyma (see Fig. 4). Therefore, we conclude that the oxygen sensitivity of cheek pouch arterioles cannot be explained by a local action of a parenchymally derived mediator.

We can also reject the hypothesis that a parenchymally derived mediator was transported in the blood because occluded aparenchymal arterioles and in vitro cannulated arterioles were oxygen sensitive. Thus, blood transport of a parenchymally derived mediator cannot explain arteriolar oxygen sensitivity in the hamster cheek pouch.

The remaining hypothesis cannot be so easily evaluated. We made several experimental attempts to exclude the possibility that the oxygen response in aparenchymal segments was partially or wholly the result of propagation. These experiments were based on the idea that the initial oxygen response might have originated with some event in parenchymal cells which then triggered a vascular response that was subsequently propagated to the aparenchymal segment. The key experiment in these attempts was the use of the covered occluded aparenchymal segment. If propagation was not involved in the oxygen response, then covering and occluding the aparenchymal segments should have totally eliminated the oxygen response, since there should have been no change in vessel \( P_{O_2} \) as suffusion solution \( P_{O_2} \) was changed. However, we observed a greatly attenuated but significant \( O_2 \) response. These data could be the result of propagation of an oxygen response initiated in uncovered portions of the vessel, or they could be the result of leakage of oxygen under the cover. Based on hemoglobin oxygen saturation measurements and observations after topical application of norepinephrine in the covered occluded aparenchymal segments, we think that it is unlikely that simple leakage of oxygen under the cover can explain our results. Thus, propagation as a cause of the oxygen response in the aparenchymal...
segments cannot be excluded as a possible explanation of the oxygen sensitivity in cheek pouch arterioles.

The signal propagated along the arterioles to the aparenchymal segments could be either changes in intravascular pressure due to upstream or down-stream vasomotor activity, or some celluarily conducted phenomenon. It does not appear likely that changes in intravascular pressure can explain the oxygen response of the aparenchymal segments, because segments occluded upstream and downstream displayed the same oxygen response.

Lidocaine was used in an effort to assess the contribution of cellular propagation to the oxygen response in aparenchymal arterioles. The use of lidocaine was premised on the earlier report that this local anesthetic blocks the propagated response of cheek pouch microvessels to acetylcholine (Duling and Berne, 1970a). Our experiments were not conclusive because the actions of lidocaine were nonspecific; arteriolar reactivities to both oxygen and norepinephrine were eliminated or greatly decreased in the presence of lidocaine. Thus, other experimental evidence must be brought to bear on the question of the possible role of a propagated response in the oxygen sensitivity of arterioles.

We infer that propagation of a response cannot explain all of the oxygen sensitivity of cheek pouch arterioles, because at least some of the isolated cannulated arterioles were oxygen sensitive and because the magnitude of the oxygen response was greatly reduced in the covered and occluded aparenchymal segments. From these observations, we conclude that propagation can at most explain a small portion of the observed oxygen response of cheek pouch arterioles.

Thus the local or blood-borne action of a parenchymally derived mediator is not required for cheek pouch arterioles to display oxygen sensitivity, and propagation of a response initiated by a mediator from the parenchyma can, at most, explain a portion of the oxygen response of these arterioles. These observations suggest that oxygen acts directly on the arterioles to initiate a response. In vivo, metabolites from the parenchyma may modulate the response of arterioles to changes in oxygen tension, but, at least in the epithelial portion of cheek pouch, such substances are not absolutely required for the arterioles to be oxygen sensitive.

Our data do not allow us to determine the cell type in the arteriolar wall responsible for sensing PO2 changes. Adventitial, medial, intimal, and blood elements were present in all preparations, and the oxygen sensitivity of the arterioles might have been related to an action of oxygen on any of these cells. Adventitial elements (e.g., nerves, mast cells, and fibroblasts), endothelium (see Chand and Altura, 1981), and blood elements (e.g., platelets and leucocytes (Lands, 1979; Samuelsson, 1981)) all interact with vascular smooth muscle, and it is known that at least some aspects of the function of nerves (Kunze, 1973) and endothelium (Stalcup et al., 1979, 1982) are oxygen sensitive in the appropriate range. Also, the oxygen sensitivity of vascular smooth muscle remains in question, with evidence both for (Coburn et al., 1979; Ebeigbe et al., 1980; Chang and Detar, 1980; Detar, 1980) and against (Pittman and Duling, 1973; Duling, 1974; Fay et al., 1977) vascular smooth muscle being oxygen sensitive in the appropriate PO2 range. Thus, any of these sites could be the primary oxygen sensor, and our data do not allow us to distinguish between them.

Whereas blood elements could be involved in the aparenchymal segments, it seems unlikely that blood is the oxygen sensor, because the oxygen sensitivity of isolated cannulated arterioles did not appear to be dependent on the amount of blood remaining in the lumens of these vessels.

The present observations on the oxygen sensitivity of arterioles might seem to conflict with previous studies from this laboratory (Pittman and Duling, 1973), and in particular, with the study performed by Duling (1974). In that study, the oxygen response of hamster cheek pouch arterioles was tested by changing the PO2 in the vicinity of arterioles in two ways: (1) locally, by applying solutions of high or low PO2 to discrete portions of arterioles with micropipettes, and (2) globally, by varying the PO2 of the suffusion solution flowing over the entire surface of the cheek pouch. Local changes in PO2 produced no consistent effect on the arterioles, whereas global PO2 changes of the same magnitude (as measured with oxygen microelectrodes) resulted in consistent, reproducible arteriolar responses. Duling concluded that arteriolar vascular smooth muscle is not particularly sensitive to oxygen, and that the oxygen reactivity of cheek pouch arterioles is secondary to the formation of a parenchymally derived mediator coupling tissue PO2 changes and vascular tone.

There are at least three possible explanations that could reconcile Duling’s observations with those from the present study. First, as noted by Duling (1974), local changes in PO2 may not have stimulated a long enough segment of vessel to elicit an arteriolar response. Summated responses can occur in cheek pouch arterioles (Duling and Berne, 1970a), and we have evidence that a portion of the oxygen response can be propagated so this hypothesis is feasible.

Second, it is possible that the oxygen sensors in the system are sparsely located on the surface or within the arteriolar wall, and that these sensors were not consistently stimulated in the experiments where PO2 was locally changed. Finally, the data might be reconciled if the oxygen sensor linking changes in PO2 with changes in arteriolar diameter were located toward the lumen of the arterioles. Blood was flowing in the arterioles studied by Duling, and it is possible that luminal PO2 was not affected when arteriolar PO2 was changed locally because of the high oxygen capacity of blood, the finite oxygen permeability of the arteriolar wall, and the relatively small area of the vessel wall exposed to PO2 changes in these experiments. Global changes in PO2 would have affected luminal PO2, and this could account...
for the difference in the responses that Duling obtained, and might reconcile that study with the present investigation. This idea is consistent with our measurements of relatively small changes in hemoglobin oxygen saturation produced by large global changes in Po2. Also, Busse et al. (1983) have reported observations that suggest that the endothelium is the site of the oxygen sensor in small arteries, and that luminal Po2 changes are much more effective in producing vasomotor responses to oxygen than are abluminal Po2 changes. Thus, data from the present study may not conflict with Duling’s findings. Further studies are required to differentiate between these hypotheses.

Some comment should be made on the low frequency of occurrence of oxygen sensitivity and the lability of the O2 response we observed in isolated cannulated arterioles. These findings could have resulted from selective preparative trauma, or the loss of some physical or biochemical component required for arterioles to display oxygen sensitivity. Perhaps the oxygen sensing mechanism is fragile and easily damaged or lost during preparation, or, because of experimental conditions, in spite of apparently good reactivity to other substances. This hypothesis is supported by observations in this laboratory that the oxygen sensitivity of in situ microvascular preparations improves markedly as the skill of the investigator improves, suggesting that the O2-sensing mechanism is fragile. Also, the oxygen sensitivity of arterial vascular strips can be greatly altered by subtle differences in experimental conditions, stretching, or the level of stimulation (Detar, 1980). Thus, the low frequency of occurrence of oxygen sensitivity in vitro may be related to a very fragile oxygen sensor.

Alternatively, if the oxygen sensors were located sparsely along arterioles, one could hypothesize that the frequency of occurrence of oxygen sensitivity observed in vitro represents the frequency of incorporation of viable oxygen sensors in the preparation. However, this hypothesis cannot explain the lability of the oxygen response we observed in vitro.

Finally, it could be that some substrate, supplied by blood, for example, is required by the oxygen-sensing mechanism. During preparation and cannulation for in vitro study, such a substrate might be greatly diluted, or even lost, resulting in an apparently labile or missing oxygen sensitivity. We cannot distinguish between these hypotheses from our data.

The relevance of our study with respect to local blood flow control might be questioned because the stimulus used (Po2 change from 15 to 150 mm Hg) was large, compared to the magnitude of the observed response (20% constriction). However, a 20% constriction in a 35-μm arteriole represents a decrease in arteriolar vascular conductance by a factor of one half. If the pressure gradient remained the same, flow in that arteriole would be cut in half. Thus, the magnitude of the response we observed is functionally significant. Also, suffusion solution Po2 is not necessarily the same as the Po2 seen by the arterioles. The Po2 in the vicinity of an arteriole is primarily determined by the Po2 of the blood in the arteriole (Duling and Berne, 1970b; Duling et al., 1979). This occurs because of the high oxygen capacity of blood, the relatively high blood flow velocity, and the finite oxygen permeability of the arteriolar wall. Thus, a large change in suffusate Po2 does not necessarily imply an equivalently large change in blood and, hence, arteriolar Po2. This hypothesis is supported by our measurements of arteriolar hemoglobin oxygen saturation (Fig. 2).

Figure 9 is a replot of the data in Figure 2 and demonstrates that the blood oxygen saturation or the blood Po2 vs. arteriolar diameter relationship is steep, indicating that these arterioles are very sensitive to changes in Po2. It is difficult to extrapolate from the cheek pouch to other tissues; however, our data suggest that oxygen must be considered as a potential mediator in the local control of blood flow.

![Figure 9. Oxygen dose-response relationship for aparenchymal arterioles in situ. Data for this figure come from Figure 2, and are expressed as means ± se, with the numbers of observations indicated above the error bars. The solid line in panel A represents the regression line fitted to the raw diameter and % saturation data for this group. The regression coefficients for the line are shown in the figure, and the regression was significant (P < 0.05). The data in panel B were obtained by estimating the blood Po2 from the hemoglobin % saturation given in panel A, using the oxygen dissociation relationship for hamster blood (Ulrich et al., 1963). The curve drawn through the mean values in panel B is the regression line in panel A converted to blood Po2 using the O2 dissociation curve.](http://circres.ahajournals.org/)

524 Circulation Research/Vol. 53, No. 4, October 1983
In conclusion, the O₂ sensitivity of hamster cheek pouch arterioles results at least in part from a local action of oxygen on the arterioles or arteriolar blood, and a portion of the response may be the result of some propagated phenomenon of parenchymal, arteriolar, or blood origin. The Po₂ in the immediate vicinity of an arteriole in vivo is primarily determined by the Po₂ of the blood in the lumen of the vessel as noted above, so that the consequences of either a direct action of O₂ on the arteriole or an action mediated through the blood are indistinguishable. If the oxygen sensitivity of arterioles in other tissues is similar to that in the cheek pouch, then changes in oxygen supply to, or oxygen demand by, a tissue that result in changes in arteriolar blood Po₂ will affect arteriolar diameter and, hence blood flow. Propagation of an oxygen-related response would allow spatial integration and, perhaps, effective recruitment of flow, dependent on the degree of oxygen lack.

This research was supported by U.S. Public Health Service Grants HL12792 and HE13437. Dr. Jackson was the recipient of U.S. Public Health Service Postdoctoral Fellowship HL60337. Dr. Jackson's present address is: Department of Physiology, Medical College of Georgia, Augusta, Georgia, 30912.

Address for reprints: Dr. Brian R. Duling, Department of Physiology, University of Virginia School of Medicine, Box 449, Jordan Medical Education Building, Charlottesville, Virginia 22908.

Received April 11, 1983, accepted for publication August 16, 1983.

References


INDEX TERMS: Oxygen • Vascular smooth muscle • Arterioles • Microvascular reactivity • Lidocaine • Local control of blood flow • Hyperemia
The oxygen sensitivity of hamster cheek pouch arterioles. In vitro and in situ studies.

W F Jackson and B R Duling

Circ Res. 1983;53:515-525
doi: 10.1161/01.RES.53.4.515

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/53/4/515