Oxygen sensitivity of vascular smooth muscle. II. In vivo studies

BRIAN R. DULING

Department of Physiology, University of Virginia School of Medicine, Charlottesville, Virginia 22901

Duling, Brian R. Oxygen sensitivity of vascular smooth muscle. II. In vivo studies. Am. J. Physiol. 227(1): 42-49, 1974. The oxygen sensitivity of vascular smooth muscle of vessels in the hamster cheek pouch was assessed by producing localized changes in the perivascular Po2 of individual arterioles. A physiological salt solution with either a high (O2 pipettes) or low (N2 pipettes) Po2 was ejected from micropipettes onto the surface of the microvessels. The responses to these localized Po2 manipulations were compared with the responses to a more widespread Po2 change induced by an increase in the Po2 of the solution flowing over the tissue surface; solution Po2 was increased from 10 to 150 mmHg. Elevation of this solution Po2 increased perivascular Po2 by an average of 10 ± 6 mmHg and caused a reduction in mean vascular diameter of 7 ± 1 μm. In contrast, local elevations of Po2 with the O2 pipettes produced very large increases in perivascular Po2 (186 ± 34 mmHg), but much smaller vasoconstrictions (1.3 ± 0.7 μm). The constrictions induced by the O2 pipettes were not different from those induced by the N2 pipettes (1.8 ± 0.9 μm); however, even though the Po2 was decreased (2.5 ± 0.6 mmHg) with the N2 pipettes, the effect of the pipettes appeared to be due to either mechanical effects or to washout of vasodilator metabolites, rather than to a change in Po2 per se. The findings suggest that the vasoconstriction associated with an increase in solution Po2 was not due to the direct, local effect of oxygen on the contractile activity of arteriolar smooth muscle cells.

Oxygen tension; flow regulation; reactive hyperemia; cheek pouch; tissue Po2

It is well known that tissue metabolic rate and blood flow are usually matched. The link which couples blood flow and metabolism has been the subject of extensive investigation but to date there is no general agreement with regard to the specific substances or processes involved. One of the earliest substances suggested as a possible mediator in this local regulatory process was oxygen (15, 20, 23). More recently, its involvement has been both supported (1, 2, 4, 5, 12, 13, 16, 18, 19, 26) and challenged (3, 14, 22).

The possible role of oxygen in local blood flow regulation would be much clearer if the ambient Po2 in association with the microvessels were known and if the O2 sensitivity of the smooth muscle cells could be determined. In previous experiments we found that arteriolar vascular smooth muscle Po2 would not likely be less than 20 mmHg; obviously, the smooth muscle Po2 cannot be greater than arterial blood Po2. In these experiments the perivascular Po2 in the microcirculation was also measured as changes were induced in the respiratory gas composition of a bathing solution, and it was found that elevations in solution Po2 produced vasoconstrictions. We can, therefore, assert that if oxygen were acting directly in the contractile response, the muscle cells would have had to be responsive to changes in oxygen tension within the range from a high level equal to systemic arterial Po2 down to a low level of approximately 20 mmHg (7-9).

There have been a number of experiments in which the O2 sensitivity of isolated portions of the large vessels has been assessed (4, 5, 11, 12, 21, 23, 25). However, since the muscle of the large vessels may not be representative of that in the arterioles, and also because of possible differences in Po2 between the interior and exterior of these tissues, the applicability of these data to the function of arterioles might be questioned. We studied the oxygen sensitivity of carotid artery vascular smooth muscle in an earlier investigation and found that a large part of the response to O2 was entirely attributable to the thickness of the tissue (91). It was found that, as tissue thickness was reduced, the limiting Po2 for muscle contraction was also reduced. The data were consistent with the hypothesis that the performance of individual smooth muscle cells would not be limited at Po2's above a few millimeters Hg, that is, substantially lower than is measured in the microcirculation.

In vivo experiments have been performed on arterioles by altering the oxygen environment of the microvessels during direct observation; both topical application of solutions with various Po2's and changes in the inspired gas have been used (8, 9, 17-19, 24, 26). The reports of the effects of such manipulations vary, but it seems clear that, in general, elevation of oxygen supply causes reduction in microvascular diameter and closure of capillaries. However, in all these experiments the oxygen tension of both vessels and parenchymal cells was changed simultaneously and the primary effect of oxygen on the vascular smooth muscle cells cannot be ascertained. It is possible that the diameter reductions were due to altered concentration of a metabolite produced by the parenchymal cells rather than to an effect of oxygen directly on the arteriolar smooth muscle.

The aim of the present experiments was to obtain an estimate of the sensitivity of the in situ arteriolar smooth muscle to local Po2 changes and to compare these findings with the observations made during arteriolar constrictions induced by elevations in suffusion-solution Po2.
METHODS

Twenty-six male golden hamsters were anesthetized initially with pentobarbital (60 mg/kg, ip). Tracheas and femoral veins were cannulated and pentobarbital was replaced as needed by urethan administered as a 50% solution (wt/vol) intravenously. The pouch was everted, spread, pinned on a Lucite pedestal, and slit longitudinally for viewing as a single layer by the methods described earlier. The pouch surface was suffused with a warm physiological saline solution having a composition (in mM) of: NaCl, 119.9; KCl, 4.7; CaCl₂, 1.6; MgSO₄, 1.2. The solution was buffered with 21 mM Tris and the pH was adjusted to 7.35. Temperature of the pouch was measured on its lower surface with a Yellow Springs telethermometer and was maintained at 37.5°C by heating the incoming fluid. Rectal temperature was maintained at 37.5°C with incandescent heating.

Suffusion-solution O₂ was normally maintained at low levels of 10–15 mmHg by equilibration of the solution in the reservoir with nitrogen. The 10–15 mmHg O₂ reflects flux of O₂ from the air over the surface of the preparation into the solution. When desired, the equilibration gas could be changed to room air to elevate suffusion-solution O₂ and thus reduce the requirement for tissue oxygen supply via vascular sources. For convenience, this will be designated as air suffusion, and use of the nitrogen-equilibrated solution will be designated N₂ suffusion. Animals were allowed to stabilize 1–2 h before experiments were begun. Vessel reactivity, tone, and maximal vessel diameter were assessed by application of a drop of acetylcholine (ACh, 5 × 10⁻⁴ M) to the suffusion solution.

Measurement of Po₂ and diameter. Oxygen tensions were measured using oxygen electrodes of the type developed by Whalen et al. (27). Electrodes had tip diameters ranging from 2 to 5 μm and were polarized with 0.7 VDC. Currents were measured with a Keithley 602 picoammeter and ranged between 10⁻¹² and 10⁻¹⁰ A. Electrodes were calibrated in suffusion solution equilibrated with gases of known composition.

Measurements of Po₂ in the tissue were made as described previously (8). Perivascular Po₂ measurements were made on the external wall of vessels with the electrodes pressed against the vessel surface, tissue measurements were made in locations up to a few hundred micrometers from the vessel studied, and the locations were chosen to lie in an area free of capillaries. These measurements thus represent the least oxygenated portions of the tissue surface. Typically these sites were 30–60 μm from a capillary.

Diameter measurements were made using a Vickers image-splitting eyepiece in conjunction with objective lenses between ×20 and ×50 magnification.

Changes in vascular smooth muscle Po₂. The perivascular Po₂ could be changed by simply elevating the suffusion-solution Po₂. However, this simultaneously altered the tissue oxygen delivery and thus may have induced vasomotor effects secondary to altered tissue metabolite production. For this reason a second method was used which permitted more localized Po₂ changes around individual arterioles. Micropipettes were filled with suffusion solution equilibrated with either N₂ (N₂ pipettes) or 95% O₂ (O₂ pipettes). Most of the pipettes had tip diameters between 4 and 6 μm. Two of the O₂ pipettes were 10 μm in diameter. The pipettes were connected to a mercury column, the height of which was adjustable from 0 to 30 cm. Each pipette was tested prior to use by placing its orifice opposite the tip of the oxygen electrode at a distance of 1–5 μm. The mercury column was then elevated and it was ascertained that Po₂ changes of the appropriate direction and magnitude could be induced by forcing fluid from the pipette. Before it was lowered onto a vessel, the zero point for the pipette was determined by lowering the mercury reservoir to a point giving zero Po₂ change. The pipettes were then positioned with a Leitz micromanipulator over suitable vessels.

In early experiments, efforts were made to measure the Po₂ distributions around the vessels at various microsuffusion rates. However, fluctuations in flow rate and the spatial variability were great enough to make this measurement unusable. The Po₂ distributions were dependent on the geometries of other vessels in the area, the amount of connective tissue present, size of the vessel being studied, and flow velocity of the suffusion solution. In general, a Po₂ quite close to the Po₂ of the solution in the pipette could be measured at the tip, and this fell off rapidly with a total distance of influence on the order of a few hundred micrometers at high flow rates from the pipette.

The large variability led to the use of an alternate method of measurement. In this method, the oxygen electrodes were positioned against the vessel wall opposite the micropipettes. The use of this position ensured that the measured changes in Po₂ gave a low estimate of the average Po₂ change over the vessel circumference and, therefore, gave a conservative estimate of the stimulus. The positions of pipettes and electrodes are illustrated schematically in Fig. 3.

RESULTS

It was found that microapplication of oxygen-rich or oxygen-poor solutions could be used to induce periarteriolar Po₂ changes with minimal changes in Po₂ at the tissue sites. With low flow rates from pipettes, Po₂’s at the tissue sites were unchanged during microapplication of fluids from either the O₂ or N₂ pipettes. In contrast to this, when suffusion-solution Po₂ was elevated by equilibrating the reservoir with air rather than N₂, there was consistently an elevation of Po₂ at both tissue and vascular sites.

When tissue Po₂ and perivascular Po₂ were measured simultaneously with two microelectrodes, a temporal separation between the Po₂ change induced at the tissue and perivascular sites could be observed. An example of this type of experiment is shown in Fig. 1. The horizontal line segments in the upper trace show inside vessel diameter. The lower two traces show arteriolar wall Po₂ and tissue Po₂. At the arrow, air was introduced into the reservoir holding the suffusion solution. There is some delay which reflects, to a large extent, transit time from the reservoir to the pouch. After this delay the tissue Po₂ began to rise and, subsequent to this, the vessel Po₂ began to rise. In five experiments of this type, the tissue Po₂ elevation preceded the vessel Po₂ elevation by an average of 42 ± 7 s.

The vessel diameter was markedly reduced by air suffusion and at the arrow in the upper trace there was a period of zero flow, which lasted about 4 min. Flow returned, but at
over variable lengths of vessel and, in general, the length was roughly proportional to the rate of efflux of fluid from the pipettes. There was no indication of the presence of any especially sensitive sites on the vessels.

Figure 2 shows a typical result obtained with an O₂ pipette. In panel A the vessel was tested initially with ACh and showed good tone and rapid responses. The perivascular PO₂ was then increased to 48 mmHg by elevating the column supplying the O₂ pipette and this level was sustained for 10 min. During this time, there was no consistent diameter change. In panel B the PO₂ was raised again to the same level, but the PO₂ change was induced by room-air suffusion rather than microapplication from an O₂ pipette. There was a marked decrease in diameter correlated with the elevation of PO₂. In addition to arteriolar constriction, all capillaries in this field closed. In panel C the periarteriolar PO₂ was reduced slightly by microapplication of a N₂ solution. Note that this produced a constriction larger than that observed with air suffusion. This will be discussed in conjunction with Fig. 3.

Diameter changes could be induced with the O₂ pipettes at high flow rates and associated high perivascular PO₂'s. Figure 3 shows the responses of two arterioles to an O₂ pipette and to air suffusion. The vessels observed are shown schematically in the upper right corner of the figure. The first portion of the record shows a three-step elevation of the mercury reservoir from 3 to 7 cm in height. At 3 cm there is an elevation in PO₂ but no change in diameter. Further increases in the fluid flow from the pipette, at 6 and 7 cm, produced additional increments in PO₂ and associated diameter reductions.
The effect of microapplication of fluids might be due to any of four different phenomena. First, the constriction might be the result of a direct effect of \( \text{O}_2 \) on the smooth muscle cells. Second, even though the \( \text{PO}_2 \) change induced by the pipettes is spatially restricted, there are tissues in the area other than the vessel whose metabolism might be altered by the local \( \text{PO}_2 \) changes. Third, there might be simply a mechanical effect due to the pressure of the fluid jet against the vessel surface. Relevant to this is the observation that at high flows, movement of the tissues could be observed when either the \( \text{N}_2 \) or \( \text{O}_2 \) pipette was used. Fourth, the flow of Ringer over the vessel surface might wash away vasodilators formed by the tissue and thus produce a constriction. Observations made during microapplication of solutions equilibrated with \( \text{N}_2 \) help to separate these possibilities.

The latter portion of Fig. 3 shows microapplication of solution equilibrated with \( \text{N}_2 \) and it can be seen that contractions similar to those induced by \( \text{O}_2 \) micropipettes were produced with \( \text{N}_2 \) pipettes even though there was either no change or a slight decrease in perivascular \( \text{PO}_2 \). As usual, air suffusion resulted in a profound constriction of both vessels. Relevant to this is the observation that high flows, movement of the tissues could be observed when either the \( \text{N}_2 \) or \( \text{O}_2 \) pipette was used. Fourth, the flow of Ringer over the vessel surface might wash away vasodilators formed by the tissue and thus produce a constriction. Observations made during microapplication of solutions equilibrated with \( \text{N}_2 \) help to separate these possibilities.

Table 1 shows the steady-state values of \( \text{PO}_2 \) changes induced by the micropipettes. Table 1 shows that the effects of microapplication of Ringer solution bore no consistent relation to the induced \( \text{PO}_2 \) changes. In the steady state, the \( \text{PO}_2 \) change induced by air suffusion was much less than that induced by the \( \text{O}_2 \) pipettes, but the diameter reduction was significantly greater (\( P < 0.05 \)).

As mentioned previously, there were frequently responses which were short-lived when the micropipettes were used. Therefore, the peak changes in diameter and \( \text{PO}_2 \) were also analyzed to make certain that no transient response to oxygen escaped detection. There was a large range of control values of both diameter and \( \text{PO}_2 \) in the vessels studied (40–12.5 mmHg, and 33–5.5 \( \mu \text{m} \), respectively), and for comparative purposes peak responses were normalized by calculating the experimental value as percent of the control value; the results are shown in Fig. 4. There was a statistically significant correlation at the 5% level between percent change in diameter and percent change in \( \text{PO}_2 \) for the 95% \( \text{O}_2 \) pipettes, but not for the other two sets of data. It should be emphasized that \( \text{PO}_2 \) was elevated by increasing the rate of flow from the micropipette, and therefore, when using the \( \text{N}_2 \) and \( \text{O}_2 \) pipettes there would have been increases in both mechanical effects and vasodilator washout coincident with larger changes in \( \text{PO}_2 \). The overall average change in diameter induced by the \( \text{O}_2 \) pipettes was not significantly different from that induced by the \( \text{N}_2 \) pipettes. During room-air suffusion, the change in diameter was significantly larger than that observed with either pipette. The \( \text{PO}_2 \) change with air suffusion was significantly larger than that observed with the \( \text{N}_2 \) pipettes and significantly smaller than that observed with the \( \text{O}_2 \) pipettes. It should be noted that in only two cases was any increase in diameter produced by the \( \text{N}_2 \) pipettes, even though perivascular \( \text{PO}_2 \) was reduced to as little as 11 mmHg.

### Table 1. Changes in oxygen tension and microvascular diameter

<table>
<thead>
<tr>
<th></th>
<th>( \text{%O}_2 ) pipette</th>
<th>( \text{N}_2 ) pipette</th>
<th>Air suffusion</th>
</tr>
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<tbody>
<tr>
<td>( \Delta \text{Oxygen tension, mmHg} )</td>
<td>186±34* (13)</td>
<td>-2.5±0.6 (15)</td>
<td>40±6.2* (14)</td>
</tr>
<tr>
<td>( \Delta \text{Vascular diameter, ( \mu \text{m} )} )</td>
<td>-1.3±0.7 (13)</td>
<td>-1.6±0.9 (15)</td>
<td>-7.3±1.1* (14)</td>
</tr>
<tr>
<td>Peak response</td>
<td></td>
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<tr>
<td>( \text{%O}_2 ) mucropipette</td>
<td>228±31* (15)</td>
<td>4.8±1.0* (12)</td>
<td></td>
</tr>
<tr>
<td>( \Delta \text{Vascular diameter, ( \mu \text{m} )} )</td>
<td>-3 0±0.8* (15)</td>
<td>-4.5±1.5* (12)</td>
<td></td>
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Values are means ± SE. Numbers in parentheses are numbers of vessels tested. * Significant difference from 0. \( P < 0.005 \). † Control diameters ranged from 33 to 5.5 \( \mu \text{m} \).
Figure 5 shows representative tracings of an experiment designed to assess further the relations between perivascular Po2 and vascular diameter. It shows a three-part record of an experiment in which substantially different perivascular Po2 patterns produced essentially identical diameter responses. In the first part of the tracing, air suffusion resulted in closure of the vessel. Before complete closure of the arteriole, the flow stopped spontaneously as indicated by the arrow. This was due to closure of a small arteriole feeding the terminal arteriole shown in Fig. 5.

After a recovery period a similar change in solution Po2 was produced, but as the peak perivascular Po2 response was approached, the mercury reservoir connected to a N2 pipette was raised and the Po2 was reduced to less than the control level. The Po2 was maintained at a low level throughout the anticipated period of Po2 change due to air suffusion. During this time, however, the diameter trace followed a pattern quite similar to that observed in the first test. In the third part of the record, the nitrogen pipette was applied early and the air suffusion was begun after a short delay. In this case, use of the N2 pipette prevented major Po2 changes until the vasoconstriction was fully developed.

Once again, the vascular diameter response was essentially identical to the first test, even though the pattern of Po2 changes was quite different from that seen with either of the first two stimuli.

DISCUSSION

These results confirm our previous findings that the arterioles of the cheek pouch respond appropriately to changes in oxygen content of the suffusion solution (7-9). As suffusion-solution Po2 is increased, the vascular bed constricts, presumably in an attempt to maintain either the oxygen tension or oxygen delivery to parenchymal cells constant. The question to which the present experiments were addressed was, Does the regulatory process involve a direct effect of oxygen on the vascular smooth muscle cells of the resistance vessels? The alternate possibility was that the responses were secondary to effects of oxygen on parenchymal tissue metabolism.

Previous in vitro work from this laboratory indicated that oxygen did not influence the contractile activity of in vitro vascular smooth muscle until Po2's were reached which are below the range recorded in the vicinity of the arterioles (21). If this is true of vessels as small as arterioles, then increased Po2 of the suffusion solution can alter blood flow only via some indirect mechanism. The experiments reported here support the idea that arteriolar vascular smooth muscle in the cheek pouch is relatively insensitive to changes in Po2. The total range of Po2 change induced by the pipettes was 466 mmHg, and over this range no demonstrable relation between Po2 and diameter was consistently observed with the three different methods of Po2 change. As can be seen in Fig. 4, the regression of percent change in diameter against percent change in Po2 gives three distinct groups of data which vary with the method used to change Po2. Furthermore, Figs. 2, 3, and 5 show several instances of clear dissociation of local Po2 and vascular diameter. Also, constrictions were not induced with micropipettes by changes in Po2 comparable to the changes in Po2 induced by air suffusion. Finally, consistent vasodilations were not induced by reducing Po2 with N2 pipettes. All these facts suggest that the regulatory changes observed during air suffusion are not the result of a local, direct action of oxygen on individual arteriolar smooth muscle cells.

It is not possible at this time to determine the cause of the constriction induced by the N2 and O2 pipettes. As pointed out previously, one might propose a priori that the constricted
tions were due to any of the following: direct effects of oxygen, local alterations in parenchymal cell metabolism, mechanical effects, or vasodilator washout. Since \N_{2} and \O_{2} pipettes produced similar diameter changes but opposite \P_{O2} changes (Table 1 and Figs. 2–5), it can be concluded that neither altered parenchymal metabolism nor a direct effect of oxygen on the smooth muscle cells was involved. The remaining two possibilities are indistinguishable on the basis of available evidence.

As would be expected, in both the peak and steady-state responses the \O_{2} pipettes induced substantially greater changes in \P_{O2} than did the \N_{2} pipettes. This was due to the fact that the difference between the \P_{O2} of the solution in the \O_{2} pipettes and the \P_{O2} of the vessel was much greater than the difference between the \P_{O2} of the \N_{2} pipettes and the vessel. \O_{2} pipettes produced increases in perivascular \P_{O2} of as much as 434 mmHg, whereas the maximum reduction in \P_{O2} produced by a \N_{2} pipette was 32 mmHg. The change in perivascular \P_{O2} induced during air suffusion was intermediate between that seen with the \N_{2} pipette and that seen with the \O_{2} pipette.

The interaction between pipettes and perivascular \P_{O2} is highlighted in Fig. 5. Inspection of the middle response shows that the perivascular \P_{O2} was reduced to the lowest levels during the period of zero flow. Also, it is apparent that the \P_{O2} rose coincident with the increase in vascular diameter which occurred at the end of the middle period. In addition, in the last test there was no detectable effect of the \N_{2} pipette when it was initially used, but the \P_{O2} fell sharply when vessel diameter and blood velocity decreased.

All of these observations reflect the fact that wall \P_{O2} of arterioles is highly dependent on the intravascular \P_{O2}. During periods of high blood flow, the small \P_{O2} difference between the fluid around the vessels and that in the pipettes is inadequate to produce major perivascular \P_{O2} changes when the \N_{2} pipettes are used. However, when the flow slows or stops, perivascular \P_{O2} is then largely dependent on the environmental (suffusion solution) \P_{O2} and is easily lowered by microapplication of the nitrogen solution.

The last portion of Fig. 5 suggests that the micropipettes are capable of preventing the altered suffusion-solution \P_{O2} from modifying the arteriolar \P_{O2}. Microapplication of low oxygen solutions does not eliminate the arteriolar response to air suffusion, however. This implies that any vasodilator metabolite coming from the tissue must be elaborated at a rate faster than it is washed away by micro-suffusion under these circumstances. This is consistent with the origin of any such metabolites from a relatively large amount of tissue both around and under the segment of arteriole being observed.

An additional qualification should be placed on the observations made with the \N_{2} pipettes. As previously indicated, constrictions are induced with both the \N_{2} and the \O_{2} pipettes. Thus, it is possible that any vasodilatation induced by the \N_{2} pipette was masked by the constriction associated with microapplication of the fluids.

At the present time it is not possible to assess the extent to which the findings reported here can be applied to other forms of regulation and to other tissues. The cheek pouch is basically an epithelial structure with striated muscle fibers surrounding its more proximal portions; both the metabolic rate and blood flow in this tissue are relatively low and comparable to the low range from resting white skeletal muscle (9). Thus the tissue might display regulatory features which are very different from those seen in a tissue in which the blood flow and metabolic rates are high, as in contracting skeletal muscle and heart. In support of the general applicability of our findings is the fact that both the epithelial and striated muscle portions of the pouch showed similar patterns in response to perivascular \P_{O2} changes. We have attempted experiments similar to those reported here on the cremaster muscle in an effort to obtain data on a more typical striated muscle vascular bed, but were not successful because the arterioles were largely buried under muscle cells and ejection of fluid from the pipettes produced an unacceptable level of tissue distortion.

In so far as the generality of our findings is concerned, it can be noted that both the in vivo experiments of the present paper and the in vitro experiments published previously (21) are consistent with the hypothesis that vascular smooth muscle is similar to other kinds of tissue in that mitochondrial respiration is not limited by oxygen until \P_{O2} falls to fairly low levels. Thus, if vessels of other tissues respond directly to oxygen, their vascular smooth muscle must display a specialized sensitivity to oxygen which has yet to be demonstrated. Also, there is the possibility that some substance produced by the tissues may modify the response of the muscle cells to oxygen (12).

There are circumstances under which oxygen might act directly in blood flow regulation, even though the present results and previous work (21) indicate that the sensitivity of both carotid artery smooth muscle and arteriolar smooth muscle is inadequate to describe the type of regulation seen here. For example, oxygen might have a direct effect on smooth muscle cells in any type of regulation which acts through a reduction in arteriolar flow to zero or to very low levels. This would occur during vascular occlusion, or perhaps at a closed precapillary sphincter lying at some distance from an arteriole as proposed by Honig (16). Under these circumstances, tissue metabolism would reduce oxygen tension to the low levels apparently required to relax smooth muscle cells and vasodilation could occur. However, the importance of such a mechanism remains to be shown, as it might be that vasodilator metabolite production in the ischemic region would dominate the response, even though oxygen could be a contributing factor.

Oxygen might also act directly to influence tension of the smooth muscle, and thus blood flow resistance, if there are thick-walled vessels in which diffusion limits \O_{2} supply of the media. In previous papers we described the quantitative relations which should obtain relating wall thickness and diffusion limitation of \O_{2} supply (8, 21). From these, it can be predicted that, given comparable tissues, diffusion limitation could occur if a class of vessels exists which has an intimal-medial thickness of 160 \mu m or greater, without vasa vasorum. To play a role in regulation of blood flow, such vessels would also have to contribute a significant fraction of the total vascular resistance of the bed. Given these circumstances, the hypothesis could be advanced that normally the tension of a fraction of the vessel wall is less than maximum because of a low \P_{O2}. When \P_{O2} of the arteriolar blood was raised, mural \P_{O2} would increase and
vasoconstriction would result. This type of influence could well explain the vasoconstriction induced in tissues by increases in blood oxygen tension (1–4, 14, 26). However, it is not consistent with the responses seen in thin-walled arterioles which are the major resistance vessels since evidence has been presented here that PO₂ in the walls of these vessels is higher than the limiting value.

It might also be argued that there are specialized O₂-sensitive muscle cells which act as pacemakers for the rest of the vessel. Certainly this is possible, but no suggestion of the presence of such cells was seen. During air suffusion, the vessels seemed to respond uniformly and no preferential segments of arteriole. Thus during microapplication of O₂-rich solutions, the local elevation of perivascular PO₂ might not have affected large enough regions to produce a response. This is possible and indeed it has been shown that the arterioles of the cheek pouch have the necessary potential to propagate responses and to function as a unit (10). The functional unit for the oxygen response would have to be quite large, however, since as was pointed out earlier, PO₂ changes extending over as much as 300 μm were induced at the high flow rates using the O₂ pipettes. This is difficult to reconcile with the very localized responses which can be induced by both vasodilators and vasoconstrictors in this tissue (10). Furthermore, this would not seem to be a very precise way of balancing flow and metabolism since it would operate in situ only when large amounts of tissue are involved.

The simplest hypothesis which would explain the arteriolar response to elevated suffusion-solution PO₂ would seem to be that the constriction arises as a result of altered release of a vasoactive substance from the parenchymal cells. This is in agreement with both the apparent lack of sensitivity of the arteriolar smooth muscle cells to oxygen and with the fact that changes in tissue PO₂ precede both the changes in diameter and changes in the arteriolar PO₂ (Fig. 1).

Figure 1 raises an important question in this regard. In Fig. 1, the vascular diameter is seen to change at a time when tissue PO₂ is well above the 0–5 mm Hg range; generally considered to include the limiting PO₂ for oxidative phosphorylation. The question which then arises is, What is the sensor for the change in tissue PO₂? There are three possible answers. First, the oxygen sensitivity of the parenchymal cells might be high or there may be special O₂ receptors located somewhere in the tissue. There is no evidence to support either of these possibilities, however. A second possibility is that some oxygen-sensitive process other than oxidative phosphorylation is responsible for the coupling between oxygen supply and oxygen demand. Again, there appears to be no evidence to indicate that this is the case. Finally, most of the vessels that supply the pouch originate on the surface being observed and the electrode is placed on the tissue surface. If the oxygen electrode is pushed down into the tissue, the measured PO₂ falls progressively to very low values of a few millimeters Hg. This region with a low PO₂ may be the one which elaborates the vasoactive substance and the substance must then be assumed to diffuse to the vessels. This seems to be the most likely explanation, but a final answer awaits a more complete description of the chemical events which couple flow and metabolism.

REFERENCES


