Estimating oxygen consumption rates of arteriolar walls under physiological conditions in rat skeletal muscle

Masahiro Shibata, Shigeru Ichioka, and Akira Kamiya

Department of Biomedical Engineering, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

Submitted 17 August 2004; accepted in final form 19 January 2005

Shibata, Masahiro, Shigeru Ichioka, and Akira Kamiya. Estimating oxygen consumption rates of arteriolar walls under physiological conditions in rat skeletal muscle. Am J Physiol Heart Circ Physiol 289: H295–H300, 2005. First published January 21, 2005; doi:10.1152/ajpheart.00830.2004.—To examine the effects of vascular tone reduction on O2 consumption of the vascular wall, we determined the O2 consumption rates of arteriolar walls under normal conditions and during vasodilation induced by topical application of papaverine. A phosphorescence quenching technique was used to quantify intra- and perivascular PO2 in rat cremaster arterioles with different branching orders. Then, the measured radial PO2 gradients and a theoretical model were used to estimate the O2 consumption rates of the arteriolar walls. The vascular O2 consumption rates of functional arterioles were >100 times greater than those observed in vitro experiments. The vascular O2 consumption rate was highest in first-order (1A) arterioles, which are located upstream, and sequentially decreased downstream in 2A and 3A arterioles under normal conditions. During papaverine-induced vasodilatation, on the other hand, the O2 consumption rates of the vascular walls decreased to similar levels, suggesting that the high O2 consumption rates of 1A arterioles under normal conditions depend in part on the workload of the vascular smooth muscle. These results strongly support the hypothesis that arteriolar walls consume a significant amount of O2 compared with the surrounding tissue. Furthermore, the reduction of vascular tone of arteriolar walls may facilitate an efficient supply of O2 to the surrounding tissue.

oxygen diffusion; vascular wall; vascular tone; vasodilation; papaverine

THE LONGITUDINAL PERIVASCULAR PO2 gradient along the arteriole, first found by Duling and Bern (6), suggested a large amount of O2 consumption by the arteriolar walls as well as a significant amount of O2 diffusion from the arteriolar network. Subsequent microvascular studies (2, 3, 7, 8, 14, 19, 20, 23, 28, 31) consistently supported the general hypothesis of these theories, i.e., the longitudinal and radial PO2 gradient could be due to the combined effects of O2 diffusion and O2 consumption by the arteriolar wall. However, the magnitude of each contribution to the arteriolar PO2 gradient remains unclear, because the O2 consumption rate of the vascular wall, calculated from these PO2 gradients, far exceeded the values obtained from endothelial and smooth muscle cell suspensions or vessel segments in vitro (7, 29, 40). The recent development of O2-dependent phosphorescence quenching microscopy (30, 36, 41–43) has allowed simultaneous determination of intra- and perivascular PO2 along the arterioles (16, 32, 35, 37, 38). These measurements also support the existence of an O2 supply to surrounding tissue and strongly emphasize the contribution of O2 consumption of vascular walls to the formation of an arteriolar PO2 gradient (13, 35, 37). Using the phosphorescence quenching technique and mass balance analysis for in vivo PO2 measurements, Tasi et al. (38, 39) clearly demonstrated that steep O2 gradients across the arteriolar wall could be explained by a high O2 consumption rate of the vessel wall itself, i.e., two orders of magnitude higher than had previously been reported by in vitro experiments (21). More recently, Friesenecker et al. (10) reported the effect of vascular smooth muscle contractions on the O2 consumption of vascular walls, in which the vasconstriction significantly increases vascular wall O2 consumption. Furthermore, the contribution of vascular smooth muscle relaxation to the vascular wall O2 consumption has been reported by Hangai-Hoger et al. (12). They found that the PO2 gradient across the arteriolar wall decreased and tissue PO2 increased during verapamil-induced vasodilation, supporting the hypothesis that the vascular wall consumes a significant amount of O2 under physiological conditions. Thus the workload of the vascular wall should be evaluated at different levels of vascular tone to better understand the O2 delivery to tissue (1, 24, 25). Many studies have measured the O2 consumption rate of endothelial and smooth muscle cells in suspension and isolated vascular segments in vitro (4, 17, 18, 21, 27), but most of these studies were conducted under nonphysiological, static conditions in the absence of normal function. Thus a method for evaluation of the O2 consumption of vascular walls in the functional states is needed.

The objectives of the present study were 1) to determine the O2 consumption rate of vascular walls in skeletal muscle arterioles under normal conditions and during vasodilation and 2) to examine the effects of vascular tone reduction on the O2 consumption rate of the vascular wall. We used phosphorescence quenching laser microscopy to determine the intra- and perivascular PO2 values of rat cremaster arterioles of several different diameters under normal conditions and during vasodilation due to a topical application of papaverine. Using the measured intra- and perivascular PO2 values, we then calculated the O2 consumption rates of the arteriolar walls on the basis of a modified “Krogh cylinder model.”

MATERIALS AND METHODS

Animal preparation. Studies were performed on seven adult male Wistar rats weighing 160–200 g. All animal procedures were approved by the University of Tokyo Animal Care and Use Committee. Animals were anesthetized with urethane (1 g/kg body wt im), and a tracheotomy was performed to facilitate spontaneous breathing. The carotid artery was cannulated so that the arterial blood pressure could

http://www.ajpheart.org 0363-6135/05 $8.00 Copyright © 2005 the American Physiological Society

H295
be measured. The jugular vein was also cannulated, facilitating injection of the intravascular phosphorescent probes and supplementary doses of anesthetic. The cremaster muscle was spread out in a special bath chamber with an optical port for transillumination, and the surface of the cremaster muscle was suffused with a 37°C Krebs solution with 5% CO₂ in 95% N₂, adjusted to pH 7.3–7.4. After a 30-min equilibration period, the suffusion was stopped, and a coverslip was placed on the muscle to prevent dehydration and hyperoxia. All animals in which the mean arterial pressure fell to <70 Torr during the experiment were excluded.

**Experimental protocols.** The intra- and perivascular PO₂ values were measured immediately past the point of origin of the arteriole from the preceding order. Orders were classified as follows: large arterioles with an inner wall diameter of ~80–120 μm branching from the central cremaster artery were designated first order (1A). Branches from 1A arterioles were designated second order (2A, 50–80 μm inner wall diameter). Third-order (3A) arterioles had an inner wall diameter <60 μm and branched from 2A arterioles. The diameter of the 3A arterioles in this study is larger than that reported by Lombard et al. (26). Although the exact reason for the different vessel sizes could not be explained, this discrepancy could have resulted from a difference in the body size of the animals or a difference in the chosen arterioles. Intravascular PO₂ was measured 30 min after the injection of a Pd-porphyrin solution (~25 mg/kg body wt) into the cannulated jugular vein. Perivascular PO₂ was then measured immediately in the vicinity of the vascular walls of the same arterioles, where they were set 20 μm from the inner wall surface to avoid uncertainty arising from our inability to precisely locate the outer vessel wall surface. We measured two intra- and perivascular PO₂ values per vessel in each rat. After the PO₂ of each order of arterioles was measured under normal conditions, papaverine (10⁻⁴ mol/l) was topically applied to the muscle surface to maintain maximum vasodilation. Under these conditions, PO₂ was measured again at the same sites under normal conditions.

**Hemodynamic changes.** The changes in the arteriolar inner-wall diameters during the topical application of papaverine were analyzed offline from video-recorded images. To determine whether blood flow rates increased during papaverine application, the relative change in volume flow in 1A arterioles during papaverine application was also monitored using a noncontact laser-Doppler flowmeter (model FLO-C1EL, Omega Wave, Tokyo, Japan), in which optical scattering from the arteriole of the laser irradiation area (~160 μm diameter) was captured through the objective lens of a microscope. All experiments in which papaverine induced a <10% increase in the diameter of the blood flow compared with normal conditions were excluded.

**Phosphorescence quenching laser microscope.** A general observation of the microcirculation was performed using a modified microscope with a ×20 long-working-distance dry objective lens (CF Plan 20×0.40 EPI ELWD, Nikon, Tokyo, Japan). The microcirculation was viewed using a charge-coupled device camera (model DHCX-107A, Sony, Tokyo, Japan) connected to a video timer (model VTG-33, For-A, Tokyo, Japan) and a videocassette recorder (model SLV-RS1, Sony), and the image was displayed on a 14-in. high-resolution television monitor (model PVM-1442Q, Sony) at a final magnification of approximately ×800. Intra- and periarteriolar PO₂ values were measured using O₂-dependent quenching of the phosphorescence decay technique described previously (32). Pd-meso-tetra(4-carboxyphenyl)porphyrin (Pd-porphyrin; Porphyrin Products) bound to bovine serum albumin was used as the phosphorescent probe for the O₂-dependent quenching. The phosphorescent probe was excited by epi-illumination using an N₂-dye pulse laser (model LN120C, Laser Photonics) with a 535-nm line at 20 Hz via the objective lens. The average optical power and pulse width of the laser were 1.2 mW and 300 ps, respectively. The diameter of the epi-illuminated tissue was 10 μm on the surface. The phosphorescent emissions from the tissue were captured by a photomultiplier (model C6700, Hamamatsu Photonics, Hamamatsu, Japan) through a long-pass filter at 610 nm. To avoid contamination of phosphorescent scattering, a 20-μm-diameter pin-hole-like aperture was placed in front of a photomultiplier. Signals from the photomultiplier were converted to 10-bit digital signals at 3-μs intervals. A total of 10 pulses was irradiated to obtain a mean phosphorescence decay curve, and the decay of the phosphorescence was mathematically fitted on the basis of the rectangular distribution model (11), expressed as follows

\[ I(t) = \exp[-(1/\tau_0 + K_p \times \text{PO}_2) I_t] \times [1 + (K_p \sigma)^2] \]

where \( I(t) \) is the light intensity at time \( t \), \( I_0 \) is the initial value of light intensity at time 0, \( \tau_0 \) and \( \tau \) are the phosphorescence lifetimes in the absence of O₂ and in the area being measured, respectively, and \( K_p \) is the quenching constant. PO₂ was determined by averaging 10 measurements. With use of this equation to calculate PO₂, the accuracy of the curve fitting was improved compared with a conventional curve fitting (11). All data with a correlation coefficient <0.900 between the measured and theoretical curves were excluded.

**Theoretical model.** The basic model used in this study to estimate the O₂ consumption rate of the arteriolar wall was as follows. The Krogh model of the capillary-tissue system for O₂ delivery in skeletal muscles was modified to suit the arteriolar vascular wall having a cylindrical geometry (15). In the present model (Fig. 1), with the assumption that the blood vessel is cylindrical, with length \( L \) and the outer and inner radii of the arterioles \( R_o \) and \( R_i \), respectively, the O₂ consumption rate per tissue volume per unit time in the arteriolar wall (QO₂) was expressed by the following modified Krogh-Erlang equation (22)

\[ \text{QO}_2 = \left( \frac{P_{o2}}{P_{o2m}} \right) \left( \frac{4 \pi D_i}{\ln(R_o/R_i) - (R_o^2 - R_i^2)} \right) \]

where \( P_{o2m} \) and \( P_{o2} \) represent PO₂ values of the outer surface of the arteriolar wall and within the arteriolar wall, respectively, and \( D_i \) and \( D_o \) represent O₂ solubility and O₂ diffusivity in the arteriolar wall, respectively. Therefore, the O₂ consumption rate of the arteriolar wall was determined by utilizing the measured intra- and perivascular PO₂ values of the arterioles. The parameters used for calculation of O₂ consumption rates of vascular walls are listed in Fig. 1. Because of uncertainty with regard to the location of the outer vessel wall boundary, the outer radius was assumed to be 10% larger than the inner radius of each respective arteriole (38). Changes in the wall thickness during vasodilation were ignored when O₂ consumption rates were calculated, because the increase in vessel diameter during papaverine-induced vasodilation was <20%.

On the basis of this model, the intravascular PO₂ generally declines in a longitudinal direction along the arteriole, from upstream to downstream. The reduction in PO₂ depends on the arteriolar blood flow rate, the O₂ diffusivity, and the O₂ consumption rate in the vascular wall. Consequently, the present model can also be used to estimate the intravascular PO₂ values downstream by utilizing the measured PO₂ and the obtained O₂ consumption rate of upstream arterioles, if the arteriolar blood flow velocity and the longitudinal distance along the arteriole downstream (from 1A to 2A and from 2A to 3A) are determined. In this simulation, the following values were used as blood flow velocities: in 1A arterioles, 10 and 15 mm/s under normal conditions and during vasodilation, respectively, and in 2A arterioles, 5 and 10 mm/s under normal conditions and during vasodilation, respectively (5). The arteriolar lengths of 10 and 5 mm were used in 1A and 2A vessels, respectively.

**Data analysis.** Values are means ± SD. Data were analyzed using a one-way ANOVA. Differences between groups were determined using a t-test with Bonferroni’s correction. Differences with \( P < 0.05 \) were considered statistically significant.

**RESULTS.** Systemic arterial PO₂, PCO₂, and pH were measured using a blood analysis system (series 2000, Diametrics Medical, St.
values of all the arterioles were significantly higher than under normal conditions, possibly because of the increased regional blood perfusion induced by papaverine, but the longitudinal and radial rate of decrease was lower than under normal conditions. Figure 3 shows O₂ consumption rates in 1A, 2A, and 3A arteriolar walls under normal conditions and during vasodilation estimated from the intra- and perivascular PO₂ data in Fig. 2. The O₂ consumption rates of the arteriolar walls under normal conditions were significantly higher than those during vasodilation: 1.87 ± 0.13 vs. 0.84 ± 0.09 (1A), 1.52 ± 0.18 vs. 0.90 ± 0.17 (2A), and 1.33 ± 0.14 vs. 1.06 ± 0.18 × 10⁻³ µmol·s⁻¹·g⁻¹. During vasodilation, O₂ consumption rates of the arteriolar walls in 1A, 2A, and 3A vessels decreased to approximately the same level. Values are means ± SD. *Significantly different from normal (P < 0.05).

**Statistically significant difference between 3 arteriole groups under normal conditions (P < 0.05).**

Fig. 3. Estimated O₂ consumption rates in 1A, 2A, and 3A arteriolar walls under normal conditions and during vasodilation. O₂ consumption rates of the arteriolar walls were significantly higher under normal conditions than during vasodilation. At rest, the O₂ consumption rate of the 1A arteriolar wall was highest and sequentially decreased significantly downstream in 2A and 3A arterioles. During vasodilation, O₂ consumption rates of the arteriolar walls in 1A, 2A, and 3A vessels decreased to approximately the same level. Values are means ± SD of 7 rats. *Significantly different from normal (P < 0.05). **Statistically significant difference between 3 arteriole groups under normal conditions (P < 0.05).
10^−2 ml·s^−1·g^−1 (3A). Under normal conditions, the O2 consumption rate of the 1A arteriolar wall, located farthest upstream, was the highest, and the O2 consumption rate sequentially decreased downstream in 2A and 3A arterioles. However, during papaverine-induced vasodilation, the O2 consumption rates of the vascular walls in the 1A, 2A, and 3A vessels decreased to approximately the same level. The estimated O2 consumption rates of the arteriolar walls under both conditions are 100–1,000 times higher than in in vitro experiments. To evaluate these results, the intravascular PO2 values of arterioles located downstream were calculated using the upstream O2 consumption rates and arteriolar PO2 values theoretically (Fig. 4). With regard to the data for the 1A arterioles under normal conditions, the estimated intravascular PO2 in the 2A arterioles was 60.4 ± 6.1 Torr, whereas the measured PO2 was 61.5 ± 6.2 Torr. Similarly, when the values of the 3A arterioles were estimated using data from 2A arterioles, the estimated intravascular PO2 was 49.7 ± 5.9 Torr, whereas the measured value was 52.6 ± 7.5 Torr. When the same calculations were performed using the vasodilation data, the estimated intravascular PO2 in the 2A arterioles was 79.4 ± 6.9 Torr, whereas the measured PO2 was 76.1 ± 8.8 Torr. Similarly, the estimated intravascular PO2 of the 3A arterioles was 70.4 ± 8.7 Torr, whereas the measured value was 69.4 ± 7.2 Torr.

DISCUSSION

The O2 consumption rates of vascular walls of different-order arterioles were quantitatively determined at different levels of vascular tone in rat cremaster muscle. The principal finding of this study is that vascular tone reduction of arteriolar walls decreases the O2 consumption rate of vascular walls. Our results also strongly support the previous findings that the arteriolar wall, under normal resting conditions, consumes a significant amount of O2 compared with the surrounding tissue, as reported by Intaglietta’s group (10, 13, 35, 37–39). Furthermore, the present study demonstrated that the O2 consumption rate is highest in the arteriolar wall of 1A vessels, which are located upstream, and sequentially decreases in a downstream direction in 2A and 3A vessels under normal resting conditions. During vasodilation induced by papaverine, on the other hand, the O2 consumption rates of the vascular walls decrease to similar levels, suggesting that the high O2 consumption rate of 1A arteriolar walls under normal conditions is likely dependent on the workload of vascular smooth muscle.

In this study, we used the O2-dependent phosphorescence quenching technique to determine the intra- and perivascular PO2 values of arterioles with different diameters in rat cremaster muscle. In vivo measurements of local PO2 with a high spatial resolution were required; therefore, we applied laser microscope technology. Use of a pulse laser facilitated excitation of the phosphorescent probe in a desired area, because the light beam can be relatively condensed, even when a low-numerical-aperture objective lens is applied (33). Our system is able to expose a diameter of 10 μm on the tissue surface when a ×20 objective lens with 0.3 numerical aperture is used. In the present study, perivascular PO2 was measured in the vicinity of the vascular walls. To avoid uncertainty arising from our inability to precisely locate the outer vessel wall surface, measuring points were set 20 μm from the inner-wall surface. The phosphorescent probe concentration is low in the arteriolar wall compared with the surrounding tissue, because the arteriolar wall has a very low permeability to albumin; thus it was considered that an error incurred by the phosphorescence from the vascular wall may be limited (10). Furthermore, it is feasible that a fall in PO2 in the surrounding tissue has a significantly shallower decay than that within the vessel wall (38). The pulse laser was also effective in terms of the fading of the phosphorescence. The pulse width of the He-Ne pulse laser used in this study was 300 ps, which is much shorter than the excitation lighting of a mercury or xenon lamp light source. Even though a total of 10 pulses were irradiated to obtain a mean phosphorescence decay curve, the total exposure time was on the order of 1 ns, suggesting that light fading had little impact on the measurements.

In this study, we attempted to measure PO2 under normal conditions and during vasodilation induced by the topical administration of papaverine (10^{-4} mol/l) in the same arterioles. We then calculated the O2 consumption rates of the vessel walls under the two conditions to examine the effect of changing vascular tone on O2 consumption of arteriolar walls. Papaverine is a nonspecific smooth muscle relaxant, and its effects are known to include 1) direct action on the smooth muscle cell membrane and inhibition of the flow of extracellular Ca^{2+} into the cell, 2) inhibition of phosphodiesterase activity and increase in intracellular cAMP content, and 3)
inhibition of oxidative phosphorylation reactions. PO2 values in the 1A, 2A, and 3A vessels obtained under normal conditions in our study were 74.2, 61.5, and 52.6 Torr, respectively, as opposed to 83.7, 76.1, and 69.4 Torr, respectively, during papaverine-induced vasodilation (Fig. 2). These PO2 values under normal conditions were consistent with measurements made using other methods, such as the microelectrode technique (6, 7) and the spectrophotometric method (34). Conversely, the PO2 values were higher during vasodilation than under normal conditions at every arteriolar site. It appeared that an increase in the regional blood perfusion induced by papaverine caused an increase in PO2.

With regard to the PO2 gradient in the microcirculation, Tsai et al. (39) recently reported in detail. They demonstrated that O2 consumption in vessel walls, especially O2 consumption by the endothelial cells, seemed to affect formation of the O2 gradient in arterioles. The results of the present study led us to the same view as that reported by Tsai et al. with regard to the magnitude of O2 consumption in the walls of the arterioles; however, the contribution of the endothelial or vascular smooth muscle to high O2 consumption is uncertain. More recently, a change in the distribution of O2 in the microcirculation during vasoconstriction was reported by Friesenecker et al. (10). They used arginine vasopressin to induce the arteriolar constriction during vasoconstriction and concluded that vasoconstriction increased vessel wall and increased intra- and perivascular PO2 would be expected.

The O2 consumption rates in the arteriolar walls estimated by intra- and perivascular PO2 ranged from 1.9 × 10−2 (1A) to 1.3 × 10−2 ml·s−1·g−1 (3A) under normal conditions (Fig. 3). A comparison of these values with the value reported for cat pial arterioles by Duling et al. (7) (2.8 × 10−2 ml·s−1·g−1) and that reported for rat mesenteric arterioles by Tsai et al. (38) (6.5 × 10−2 ml·s−1·g−1) showed that our values were slightly lower but were of the same order of magnitude. With regard to the physiological data used to estimate the O2 consumption rates, Tsai et al. used 1.7 × 10−5 cm3/s for O2 diffusivity in the vessel wall and 2.1 × 10−5 ml·g−1·Torr−1 for O2 solubility in the vessel wall; consequently, there were no major differences. The ratio of wall thickness to vessel diameter was 10%. On the other hand, compared with the data obtained from endothelial and smooth muscle cell suspensions or vessel segments in vitro (4, 17, 18, 27), many of the values were on the order of 10−4–10−5, or 100–1,000 times lower than those of the present study and >10 times lower than even the vasodilation data in this study. The fact that the suspensions and segments are not subject to normal in vivo conditions may explain the lower O2 consumption.

Furthermore, to evaluate the appropriateness of our estimation, we used the O2 consumption rates we obtained to calculate the intravascular PO2 of downstream arterioles and measure intravascular PO2 of upstream arterioles (Fig. 4). As a result of calculations based on the data for the 1A arterioles, the estimated intravascular PO2 in the 2A arterioles was consistent with the measured PO2 (60.4 vs. 61.5 Torr). Similarly, calculation of the value of the 3A arterioles with use of the 2A data resulted in an estimated value that was consistent with the measured value (49.7 vs. 52.6 Torr). Moreover, when the same simulations were performed using data obtained during vasodilation, a high consistency between the estimated value and the measured value was confirmed for 2A and 3A vessels: 79.4 vs. 76.1 Torr (2A) and 70.4 vs. 69.4 Torr (3A). These simulations confirmed the validity of the estimated O2 consumption rates in the arteriole walls.

Many studies have used a theoretical model to analyze O2 transport to tissues during exercise and under normal conditions (15), but none of these reports have taken into account O2 consumption in the vessel wall. The O2 consumption rates of skeletal muscle observed in such studies were 2–4 × 10−5 ml·s−1·g−1 at rest, whereas the O2 consumption rate of the arteriolar walls obtained in the present study was 1–2 × 10−2 ml·s−1·g−1 under normal conditions. If it is assumed that the volume ratio of the arteriolar walls to the skeletal muscle tissue was 0.7% (9), the O2 consumption rate of the arteriolar walls was ~500 times higher than that of the skeletal muscle tissue, resulting in the arteriolar walls consuming an amount of O2 equivalent to, or greater than, that consumed by the skeletal muscle tissue.

In conclusion, ~100 times more O2 is consumed by functional arteriolar walls than by the vascular segments or vascular cell suspensions measured in in vitro experiments, and the O2 consumption rates of the arteriolar walls are significantly higher under normal conditions than during vasodilation. The highest O2 consumption rate of the 1A arteriolar walls under normal conditions suggests that the vascular O2 consumption is dependent on the workload of vascular walls. These results strongly support the hypothesis that the arteriolar wall consumes a significant amount of O2 compared with the surrounding tissue. Furthermore, the reduction of vascular tone of arteriolar walls may facilitate an efficient supply of O2 to the surrounding tissue.

GRANTS

This study was supported by Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research 15300156.

REFERENCES


