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Experimental and theoretical studies of oxygen gradients in rat pial microvessels

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Abstract

Using modified oxygen needle microelectrodes and intravital videomicroscopy, measurements were made of tissue oxygen tension (PO_2) profiles near cortical arterioles and transmural PO_2 gradients in the pial arterioles of the rat. Under control conditions, the transmural PO_2 gradient averaged 1.17 ± 0.06 mm Hg/μm (mean ± s.e., $n = 40$). Local arteriolar dilation resulted in a marked decrease in the transmural PO_2 gradient to 0.68 ± 0.04 mm Hg/μm ($P < 0.001$, $n = 38$). The major finding of this study is a dependence of the transmural PO_2 gradient on the vascular tone of the pial arterioles. Using a model of oxygen transport in an arteriole and experimental PO_2 profiles, values of radial perivascular and intravascular O_2 fluxes were estimated. Our theoretical estimates show that oxygen flux values at the outer surface of the arteriolar wall are approximately 10^{-5} mL O_2/cm^2 per sec, independent of the values of the arteriolar wall O_2 consumption within a wide range of consumption values. This also means that PO_2 transmural gradients for cerebral arterioles are within the limits of 1 to 2 mm Hg/μm. The data lead to the conclusion that O_2 consumption of the arteriolar wall is within the range for the surrounding tissue and O_2 consumption of the endothelial layer appears to have no substantial impact on the transmural PO_2 gradient.

Keywords

cortical microvessels; O_2 transport model; oxygen microelectrodes; tissue PO_2 profiles; transmural PO_2 gradient

Introduction

Oxygen molecules leaving microvessels diffuse along concentration gradients that are established by the spatial distribution of the vessels and the rate of oxygen consumption in the vascular wall and surrounding tissue. Capillaries are not the only source of oxygen to

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tissue (Duling and Berne, 1970). Arterioles and venules also supply oxygen to tissue and play a significant role in diffusional O_2 exchange between various segments of microvasculature. Extensive experimental studies have shown that arterioles could supply up to 50% (or even above) of all oxygen demand in resting skeletal muscle (Swain and Pittman, 1989; Ellsworth and Pittman, 1990). In cerebral tissue, arterioles supply up to 20% of all consumed oxygen (Vovenko, 1997, 1999).

The role of the wall in microvessels has been the topic of several studies, as it participates in the exchange of gases between blood and tissue. Although some studies point to a very high consumption of O_2 by the endothelial and smooth muscle cells of the wall (Shibata *et al*, 2005; Tsai *et al*, 1998, 2003), other studies suggest a consumption rate closer to that of the surrounding parenchymal tissue (Golub *et al*, 2007; Pittman *et al*, 2005; Vadapalli *et al*, 2000). Various experimental methods have been developed that make it possible to conduct PO_2 measurements in the lumen (intravascularly), or on the outer wall of microvessels and in the surrounding tissue (Wilson *et al*, 1991; Ivanov *et al*, 1982; Duling *et al*, 1979). Using these methods, the transmural gradients of PO_2 have been measured on cat pial arterioles (Duling *et al*, 1979), rat mesenteric arterioles (Tsai *et al*, 1998), rat cremaster muscle (Shibata *et al*, 2001, 2005), and other tissues (Santilli *et al*, 2000; Crawford and Cole, 1985). These data vary significantly, from 1 to 10 mm Hg/μm and above. Such large values of transmural PO_2 gradients led to the hypothesis of a very high consumption rate of O_2 by endothelium and/or smooth muscle cells (Tsai *et al*, 1998, 2003). If this hypothesis is validated, it might result in significant revision of the current concept of oxygen transport to tissue and would require taking into consideration additional oxidative metabolism in the neighborhood of the blood–tissue interface.

The main goal of the present study was to test the hypothesis of a large transmural PO_2 gradient across the blood–tissue interface. We have studied transmural PO_2 gradients on the arterioles of rat brain cortex, a tissue with high oxidative metabolism and, consequently, high blood flow. Using these data, we performed theoretical estimates of O_2 consumption in the vascular (arteriolar) wall and estimated transmural PO_2 gradients have been assessed. In addition, using oxygen microelectrodes of a special design, we measured transmural PO_2 gradients in control and vasodilated pial arterioles. Our experimental results and theoretical estimates show that transmural PO_2 gradients for rat cortical arterioles are similar to those in surrounding tissue and are in the range of 1 to 2 mm Hg/μm under control conditions and below 1 mm Hg/μm in dilated vessels.

Materials and methods

Animals and Surgical Procedures

All the experiments were performed in male Wistar rats (Pavlov Institute of Physiology vivarium) weighing 250 to 320 g. The experiments were performed in accordance with ethics regulations of The Pavlov Institute, which are in agreement with the European Communities Council Directive (86/609/EEC) for the care and use of laboratory animals. Animals were initially anesthetized by an intra-peritoneal injection of sodium pentobarbital (60 mg/kg) and then by supplemental doses of 10 to 12 mg/kg per h. They were placed on a Plexiglas platform and the head was fixed using a special teeth rod mounted on a ball-and-socket joint. A tracheostomy was made to ensure proper spontaneous ventilation. A polyethylene catheter (PE-90) was inserted into the femoral artery and vein for collecting blood samples, for gas analysis (ABL-330, Radiometer, Denmark), for measurement of mean arterial pressure, and for blood and solute injections.

A 4 × 8 mm opening was made on the right parietal lobe of the animal's scull. The dura mater was accurately removed and the exposed brain cortex was superfused with a solution

of the following composition (in mmol/L): NaCl 118, KCl 4.5, $CaCl_2$ 2.5, $KHPO_4$ 1.0, $MgSO_4$ 1.0, $NaHCO_3$ 25, and glucose 6. The solution was equilibrated with a gas mixture of 5% CO_2/5% O_2/balance N_2 and its pH was adjusted to 7.38±0.05 at 37°C.

Experimental Setup

The design and layout of the setup has been described elsewhere (Vovenko, 1999). Briefly, the microscope and 3D-manipulated stage were mounted on a massive metal beam to minimize vibrations and displacements of the microelectrode during PO_2 measurements on microvessels. Any displacements of the animal relative to the microscope were performed using the stage under the microscope. A PO_2 microelectrode was manipulated by a 3D-micromanipulator that was mounted on the objective of the microscope (Figure 1). The microelectrode tip was positioned at the center of the frontal lens of the objective. A thermostabilizing system maintained a constant temperature (37°C±1°C) of the animal's body, the objective of the microscope, and the calibration and superfusion solutions.

Visualization—The microvasculature of the brain cortex was visualized using a microscope LUMAM-K1 (LOMO, Russia) equipped with contact lens epiobjectives (×10/0.30, × 20/0.60). For visualization, the frontal lens of the objective was put in immediate contact with the brain tissue (no compression, the gap between lens and brain less than 10 μm). Focusing of the microscope was performed without displacement of the objective relative to tissue using a special lens, moved along the optical axis inside the microscope tube. The microscopic image was viewed on a TV monitor and, at the same time, on a PC monitor using a video camera (PIH-576, Taiwan) and capture card (Pinnacle Deluxe). The linear dimensions (microvascular luminal diameter, thickness of the vascular wall, and distance to the microelectrode tip) were determined during offline analysis using frame capture and a special screen caliper. The error of linear measurements was approximately ±2 μm.

Oxygen microelectrodes—Platinum oxygen microelectrodes with a tip diameter including glass insulation of 3 to 4 μm and a recess depth of 8 to 12 μm were used in the study. The polarographic current in physiologic solution saturated with room air at 37°C ranged from 5×10^{-11} to 9×10^{-11} A. Residual current was negligible. The sensitivity of the microelectrodes to fluid convection was tested by measuring the polarographic current in a stirred and unstirred solution. Convection was produced using a magnetic stirrer (linear velocity of the solution at the microelectrode tip was approximately ~100 mm/s). Oxygen microelectrodes with a recess of $l/d = 8$ to 10 showed an increase in current during the stirring of less than 2% to 3%, if any. The microelectrodes used in this study revealed a small 'calibration error' too, that is, they were not sensitive to variations in O_2 diffusion coefficient in the calibration and measurement media (Buerk, 2003; Craw-ford and Cole, 1985; Schneiderman and Goldstick, 1978). The microelectrode was calibrated several times during an experiment. To minimize measurement error before measurements, the electrode tip was inserted into the brain tissue or placed into the venular lumen for several minutes. Then, the electrode was placed into the calibration solution again. This procedure was repeated several times until a stable level of electrode sensitivity to oxygen was attained.

Experimental Protocol

The tissue was allowed to stabilize for a period of at least 10 to 15 mins before the acquisition of PO_2 data. In Series 1, measurements of radial tissue PO_2 profiles near the walls of rat precortical arterioles were performed. In Series 2, measurements of transmural PO_2 gradients were performed on rat pial arterioles. Group 1 of Series 2 consisted of transmural PO_2 measurements in control pial arterioles, whereas Group 2 of Series 2 consisted of transmural PO_2 measurements on dilated pial arterioles.

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Measurements of radial PO_2 profiles in the vicinity of precortical arterioles (series 1)—To record a tissue PO_2 profile, representing oxygen diffusion across the wall of an arteriole, the PO_2 microelectrode was positioned so that the nearest large microvessel (on the course of the electrode track) was at least 70 to 80 μm away. The PO_2 microelectrode was positioned perpendicularly to the wall of the selected arteriole. PO_2 was then measured at the wall surface and at distances of 10, 20, 30 μm and so on from the vessel wall, sequentially (Figure 2). For reproducible measurements, the PO_2 microelectrode was again brought to the surface of the outer wall to recheck the values obtained.

Measurements of PO_2 transmural gradients on pial arterioles in control (series 2, group 1)—For assessing the transmural PO_2 gradient, it is necessary to know PO_2 values on the outer and inner wall of the arteriole and the wall thickness. The transmural PO_2 gradient was defined as the difference of intravascular and outer wall PO_2 relative to the displacement of the electrode (Δl) defined as the distance from the outer wall surface to the edge of the cell-free plasma layer and red blood cell core in the arteriolar lumen. Δl was measured offline during analysis of the recorded photomicrographs. Δl could be used also as an estimate of the arteriolar wall thickness ΔL (it exceeds wall thickness by the thickness of the cell-free plasma layer) used in the mathematical analysis. Cell-free plasma layer thickness has been measured in arterioles (Kim *et al*, 2007). To measure the transmural PO_2 gradient, the microelectrode tip was positioned on the outer surface of the wall in the midplane of an arteriole. An image of the field was captured and oxygen tension was recorded. The microvessel wall was punctured by quickly moving the microelectrode into the wall using a micromanipulator (Figure S1). Intravascular readings of PO_2 were relatively stable during the first 5 to 6 secs after penetration of the vessel. Then, thrombus formation began at the microelectrode tip and a progressive decline of PO_2 occurred. Therefore, all intravascular PO_2 measurements were obtained during the first 5 to 6 secs after wall puncture, when thrombus size was negligible and it did not disturb blood flow in the arteriole. Then, the microelectrode was withdrawn from the vessel to perform a calibration.

Measurements of transmural PO_2 on pial arterioles during local vasodilation (series 2, group 2)—The vasodilator agent (~2×10^{-7} mmol/L sodium nitroprus-side in physiologic solution) was administered into the perivascular zone of the arteriole using a glass micropipette of diameter 25 to 30 μm (Figures 1C–1E). Transmural PO_2 measurements on the dilated arteriole were conducted according to the same protocol as above for the control series.

Data Analysis

All data are presented as mean ± s.e. All calculations and graphics were made using the program OriginPro ver. 7.5 (OriginLab Corp.).

Results

Tissue PO_2 Profiles (Series 1)

Sixteen male Wistar rats (250 to 280 g body weight) were used in the first series of experiments to measure radial PO_2 profiles in the vicinity of precortical arterioles.

Blood gas parameters of systemic blood were as follows: pH = 7.351 ± 0.002; PCO_2 = 37.6 ± 1.2 mm Hg; PO_2 = 78.7 ± 2.2 mm Hg (n = 19). Mean arterial pressure in the experiments of Series 1 averaged 126 ± 5 mm Hg (n = 57).

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Tissue radial PO_2 profiles around precortical arterioles of the rat measured using oxygen micro-electrodes are shown in Figures 2D–2F. During measurements, the microelectrode tip was positioned within the brain tissue. The data showed that a marked decrease in tissue PO_2 was observed in the region near the arteriolar wall (within a tissue zone of 20 to 30 μm). The PO_2 profiles were more shallow farther from the arteriole.

Transmural PO_2 Gradients (Series 2)

A total of 31 rats (230 to 250 g body weight) were used in the second series of experiments to explore transmural PO_2 gradients in rat pial arterioles under control (Group 1, 17 animals) and vasodilated conditions (Group 2, 14 animals). Blood gas parameters of systemic arterial blood of both groups of animals were as follows: pH = 7.334 ± 0.006, PCO_2 = 43.7 ± 0.5 mm Hg, PO_2 = 87.3 ± 1.3 mm Hg ($n = 34$). Mean arterial pressure in Series 2 averaged 136 ± 2 mm Hg ($n = 86$).

To measure the transmural PO_2 gradient, the microelectrode tip was brought close to the outer arteriolar wall and the control level of PO_2 was recorded. Then, the microelectrode was moved into the arteriolar lumen and intra-vascular PO_2 was recorded. The relationship between Δl and arteriolar luminal diameter is presented in Figure 3A. The relationship between ΔPO_2 and the corresponding Δl is presented in Figure 3B. The slope of the regression line in Figure 3B is numerically equal to the transmural PO_2 gradient in rat pial arterioles (1.17 ± 0.06 mm Hg/μm, $n = 40$). Figure 3C shows the relationship of the transmural PO_2 gradient and measured arteriolar luminal diameter.

The same measurements were performed on dilated arterioles to assess whether vascular tone has an impact on the transmural PO_2 gradient in rat pial arterioles. The results of Series 2, Group 2 are presented in Figures 3D–3F. The transmural PO_2 gradient in dilated arterioles was 0.68 ± 0.04 mm Hg/μm ($P < 0.001$, $n = 38$).

Discussion

We have presented experimental measurements of tissue PO_2 profiles around rat cortical arterioles and transmural PO_2 measurements in pial arterioles using specially designed fine-tip O_2 microcathodes. Our results showed that under control conditions radial and transmural PO_2 gradients did not exceed 2 mm Hg/μm. In dilated arterioles, transmural PO_2 gradients are ~0.7 mm Hg/μm. Our theoretical estimates showed that oxygen flux values at the outer surface of the arteriolar wall are around 10^{-5} mL O_2/cm^2 per sec, independent of the values of the arteriolar wall O_2 consumption within a wide range of consumption values. This also means that transmural PO_2 gradients for cerebral arterioles are within the limits of 1 to 2 mm Hg/μm.

In this study, attention was focused on O_2 diffusion through the arteriolar wall—an interface between blood and tissue. Arterioles do play a significant role in oxygen exchange between these two compartments. Arterioles are powerful sources of O_2 to tissue (high PO_2, blood flow). Nerve cells located nearby arterioles might favor under the conditions of restricted O_2 supply. In addition, arterioles take part in diffusional oxygen exchange of respiratory gases between venules and capillaries. Direct PO_2 measurements showed that anatomical capillaries deliver ~60% of all O_2 consumed by brain tissue, whereas cortical arterioles of diameter 7 to 20 μm deliver approximately 20% and arterioles of diameter 30 μm and larger deliver less than 10%. The data imply that cerebral microvasculature of caliber 20 μm is a *primary* site of gas transfer between the blood and the tissue (Vovenko, 1997, 1999).

There are a few data in the literature concerning measurements of transmural PO_2 gradients in brain arterioles. Duling *et al* (1979), using Whalen-type oxygen microcathodes in cat pial

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arterioles, found transmural PO_2 gradients of ~0.91 mm Hg/μm. Our measurements of transmural PO_2 gradients are smaller than those reported in other tissues using the optical phosphorescence quenching method (Tsai *et al*, 1998, 2003). This discrepancy might be because of the differences in the tissues studied and differences in the methodology used. Several studies point out that extravascular PO_2 measurements using the phosphorescence quenching method might contain a measurement artifact—underestimation of the actual tissue PO_2 because of an oxygen consumption artifact (O_2 consumption caused by the excitation flash impulse) (Golub and Pittman, 2005).

Shibata *et al* (2001, 2005), using the phosphorescence quenching method, measured the decrease in PO_2 in the arterioles of rat cremaster muscle and found that the PO_2 difference was approximately 15 to 20 mm Hg over a distance of 10 to 12 μm, so that the measured transmural PO_2 gradient was within 1 to 2 mm Hg/μm. A pronounced PO_2 difference (40 to 45 mm Hg) over a distance of 2 μm was found in the rabbit infra-renal aorta using a microelectrode technique (Santilli *et al*, 2000). This steep decrease in PO_2 over such a small distance, however, could be because of tissue compression/distortion during microelectrode penetration of the dense tissue (Crawford and Cole, 1985).

It is worth noting that the oxygen microelectrode technique is probably the only method enabling local PO_2 measurements in three different compartments in the brain cortex: tissue (extravascular), vascular wall (perivascular), and vessel lumen (intravascular). The method of phosphorescence quenching is applicable for intravascular (intravascular infusion of phosphor) and extravascular (topical application of phosphor) measurements only, because of the practical impermeability of the blood–brain barrier for extravasation of the albumin-bound phosphorescence probe.

Despite the advantages indicated above, the use of oxygen microelectrodes has a number of limitations. The first limitation is because of the invasive nature of the measurements. The scull is open (no closed cranial window), making the cortical tissue susceptible to the development of edema. However, the duration of the experiments was within 60 to 90 mins, after opening of the skull, and no significant edema was observed during this time.

It is extremely difficult to penetrate the wall of an arteriole of diameter 20 to 25 μm, even using thin and specially sharpened microelectrodes (with tip diameter including glass insulation ~3 μm). This is the reason why all our data on transmural gradients were obtained on microvessels of larger caliber (the data in Series 1 on smaller vessels did not involve puncturing the arteriolar wall).

From a methodological point of view, the intra-vascular PO_2 measurements represented a serious and difficult task. First of all, the puncture should induce only minimal injury of the arteriolar wall. The microelectrode tip diameter should be small (2 to 4 μm) and the shaft taper of the tip should be 3° and be sharpened similarly to a syringe needle (sharpening angle ~15° to 18°). The angle of beveling of the tip plays a critical role in minimizing trauma to the wall (even for microelectrodes with a tip diameter of 3 to 4 μm). In addition, the polaro-graphic current of the microelectrode should not depend on fluid convection (for correct measurements in flowing blood). The microelectrode readings should not be dependent on the O_2 permeability coefficient of the measurement and calibration media. Finally, the calibration characteristics of the O_2 cathodes should be relatively stable and reproducible. The oxygen microelectrodes used in this study complied with the above requirements.

Dilation of the arteriolar wall resulted in a decrease of the transmural PO_2 gradient to 0.68 ± 0.04 mm Hg/μm. This means that the vascular tone and, accordingly, the level of oxidative metabolism of the smooth muscle cells could have an impact on the transmural PO_2

gradient. It is possible that endothelial cells also contribute to the observed decrease in oxygen tension in the arteriolar wall. Nevertheless, our data do not provide support for the hypothesis of an important role for the endothelium in the transmural PO_2 gradients in arterioles.

Experimental measurements (Vovenko, 1999) yield an intravascular O_2 flux J_i of the order of 10^{-5} mL O_2/cm^2 per sec (Figure S2). Our theoretical estimates of J_i are consistent with those based on measurements when the PO_2 gradients are within 1 to 2 mm Hg/μm. Calculation of J_i in individual vessel segments also supports a value of J_i~10^{-5} mL O_2/cm^2 per sec. In addition, a majority of the computed values of J_i in microvessels, compiled by Vadapalli *et al* (2000), and based on *in vivo* measurements of longitudinal hemoglobin oxygen saturation or PO_2 gradients in arteriolar segments, are ~10^{-5} mL O_2/cm^2 per sec, consistent with those based on the observations.

The estimate of O_2 flux from the rat cremaster muscle (Shibata *et al*, 2005) results in J_i values of an order of magnitude higher than that in the present measurements. This estimation is based on morphologic assumptions about vessel length; however, they have reported oxygen gradients between 1 and 2 mm Hg/μm. In their analysis, it was assumed that the entire O_2 flux was consumed by the wall and, accordingly, they calculated an O_2 consumption in the wall that was 100 times higher than in the tissue.

For the pial arteriolar network in rats, the global estimate also yields a value of $J_i = 1.2 \times 10^{-5}$ mL O_2/cm^2 per sec (Figure S3). Using morphologic data for the rat mesentery network (Pries *et al*, 1990), the computed value of J_i is 1.03×10^{-5} mL O_2/cm^2 per sec. Global estimates of intravascular flux for the pial and mesenteric arteriolar networks in rats support a value of $J_i \sim 10^{-5}$ mL O_2/cm^2 per sec. However, global estimates of J_i for the rat spinotrapezius muscle with two sets of morphologic data for that arteriolar network (Engelson *et al*, 1985) are 3.026×10^{-4} and 9.85×10^{-5} mL O_2/cm^2 per sec. The foregoing discussion supports theoretical estimates of $J_i \sim 10^{-5}$ mL O_2/cm^2 per sec, consistent with the observations when oxygen gradients are within 1 to 2 mm Hg/μm.

In summary, theoretical estimates based on experimental data of tissue PO_2 profiles around rat cortical arterioles and direct measurements of transmural PO_2 gradients on pial arterioles showed that the decrease in PO_2 across the arteriolar wall is within 1 to 2 mm Hg/μm under control conditions and approximately ~0.7 mm Hg/μm under vasodilated conditions. The data presented lead to the conclusion that the O_2 consumption of the arteriolar wall is within the range for the surrounding tissue and that the O_2 consumption of the endothelial layer, apparently, does not have a substantial impact on the transmural PO_2 gradient.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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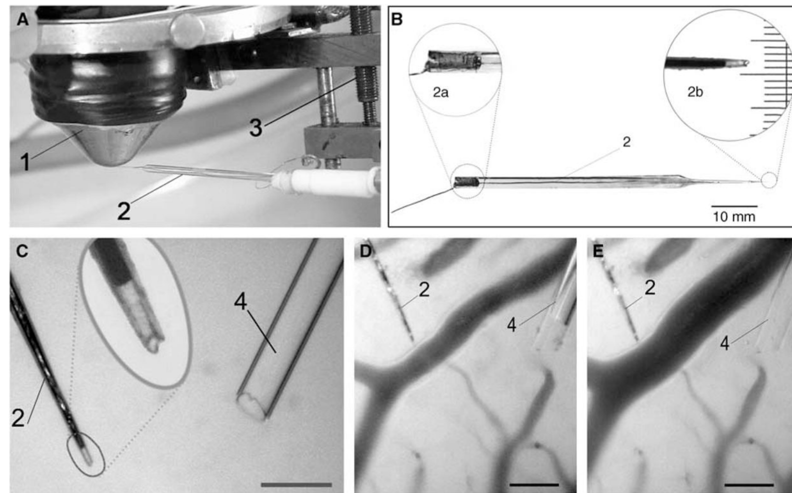

Figure 1.

Local measurements of oxygen partial pressure using a fine-tip oxygen microelectrode. (**A**) Tip of the microelectrode (2), mounted on a 3D-micromanipulator (3), is positioned in the center of frontal lens of the contact epiobjective (1) of the microscope. (**B**) Overview of the polarographic oxygen microelectrode used in the study. 2a—enlarged image of the copper wire fixed relative to a glass body using a small bit of quick-setting glue. 2b—photomicrograph of the microelectrode tip: the tip diameter including glass insulation, 4 μm; recess, 8 μm; scale, 3 μm. (**C**) Measurements of transmural PO_2 gradient in dilated pial arteriole. Oxygen microelectrode (2) with a tip diameter of 4 μm and a recess of 10 μm and a glass micropipette (4) with a tip diameter of 27 μm is shown; scale, 50 μm. (**D**) The microelectrode tip is positioned on the outer arteriolar wall before applying the vasoactive agent. Arteriolar luminal diameter is 30 μm. (**E**) The microelectrode tip is positioned on the dilated arteriolar outer wall. Arteriolar luminal diameter is 46 μm; scale, 50 μm.

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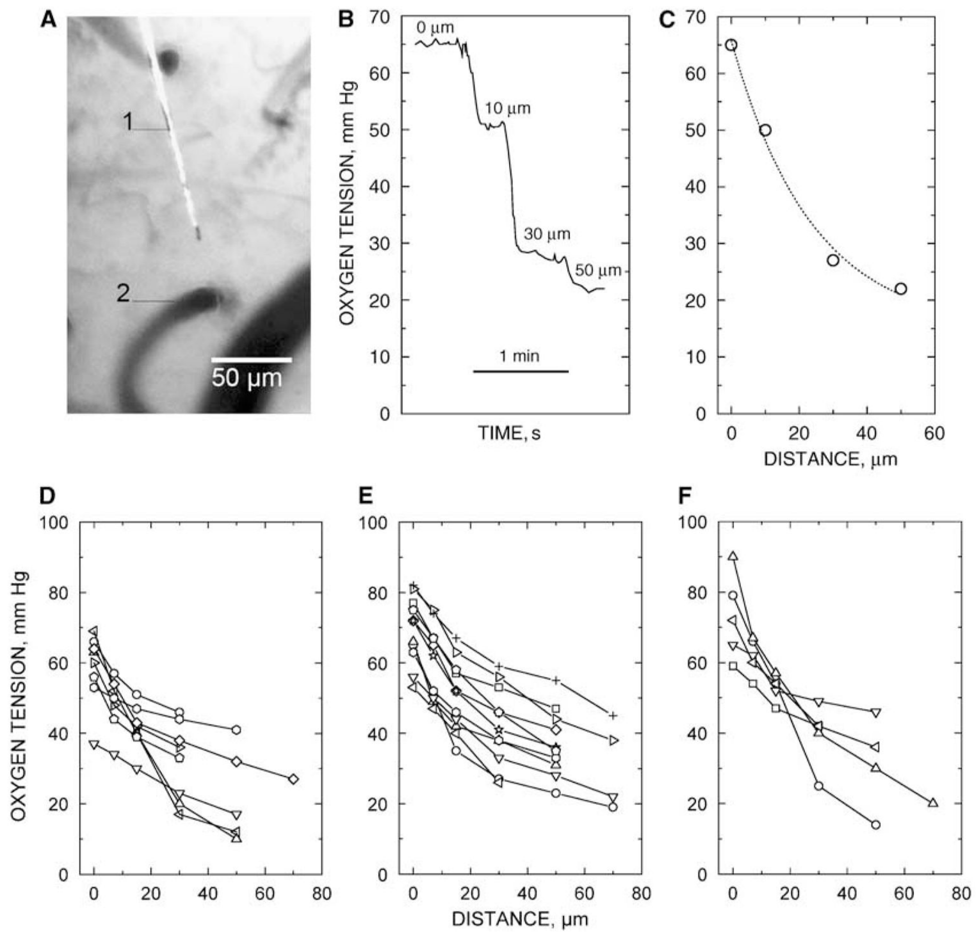

Figure 2.

Measurements of tissue PO_2 profiles near precortical arterioles of the rat. (**A**) The microelectrode tip (1) is 30 μm from the precortical arteriole (2) of luminal diameter 20 μm. Scale, 50 μm. (**B**) Example of recording of PO_2 radial profile near a cortical arteriole; scale, 1 min. (**C**) Graphical representation of the PO_2 profile. (**D**, **E**, **F**) Measurements of radial PO_2 profiles on arterioles with a luminal diameter of 10, 20, and 30 μm, respectively. Each symbol represents an individual value. The same symbols on the graphs are independent.

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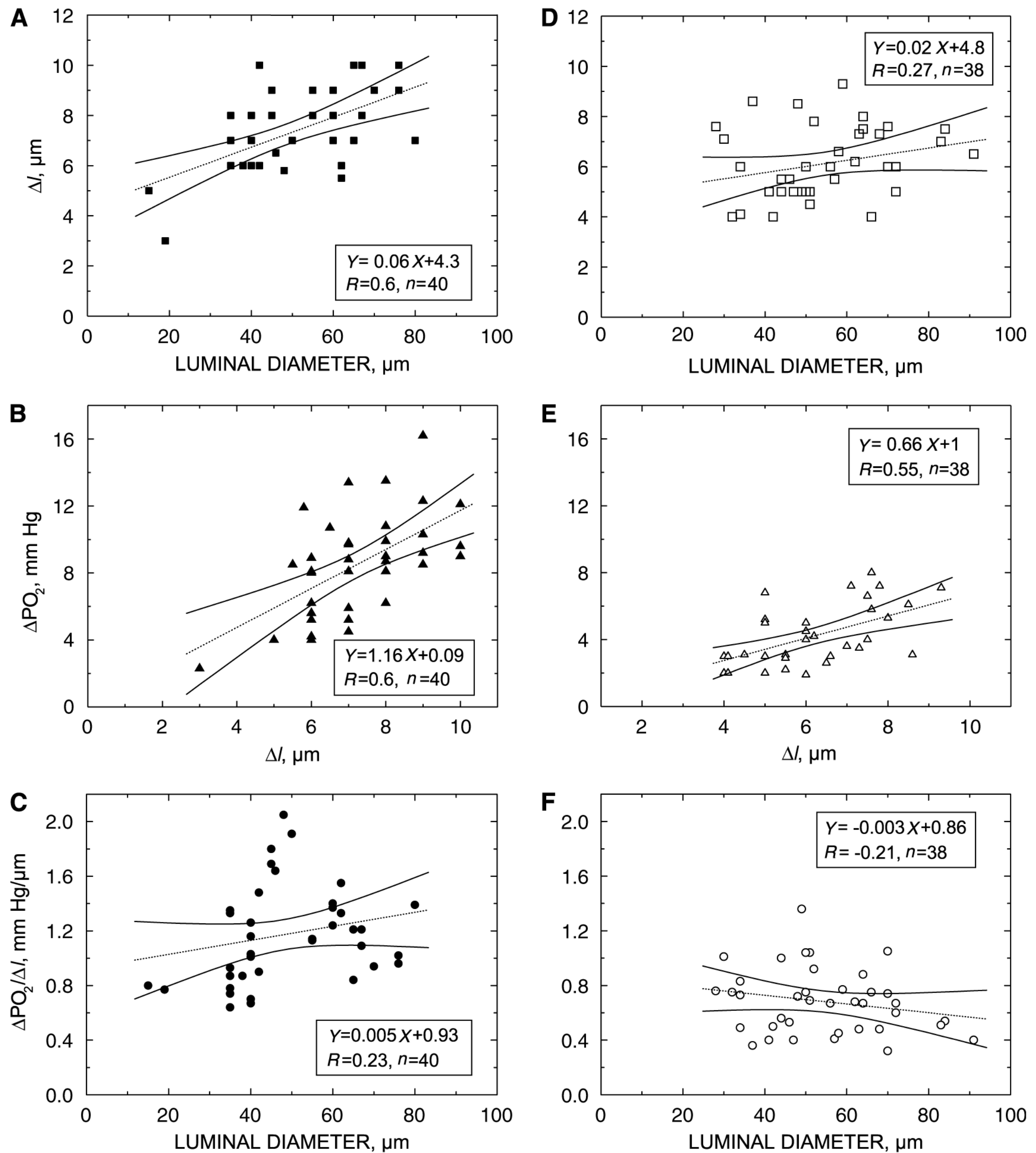

Figure 3.

Results of transmural PO_2 measurements in rat pial arterioles during control (**A** to **C**) (Series 2, Group 1) and dilation (**D** to **F**) conditions (Series 2, Group 2). (**A**, **D**) Relationship between Δl and arteriolar luminal diameter. Δl is the distance from the outer wall surface to the edge of cell-free plasma layer and red blood cell core in the arteriolar lumen. Δl could be used as a measure of arteriolar wall thickness (it exceeds the true wall thickness by the depth of cell-free plasma layer). (**B**, **E**) Relationship between PO_2 increase during penetration of the arteriolar wall (ΔPO_2) and the corresponding displacement of the microelectrode (Δl) for control and dilated arterioles, respectively. (**C**, **F**) Relationship of the transmural PO_2 gradient to the arteriolar luminal diameter for control and dilated arterioles, respectively. *R*, correlation coefficient; *n*, number of measurements. 95% confidence intervals for the regression line are shown by solid lines.