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ORIGINAL ARTICLE

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Distribution of oxygen tension on the surface of arterioles, capillaries and venules of brain cortex and in tissue in normoxia: an experimental study on rats

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Abstract The distribution of oxygen tension (PO_2) was studied in normoxia on the surface of arterioles, capillaries and venules of rat brain cortex, both longitudinally and in tissue radially from the wall of microvessels. Along the arteriolar tree, PO_2 decreased from 81.2±6.2 mmHg (mean±SD) on 1°A (first-order branch) arterioles to 61.5±12 mmHg on 5°A arterioles. Transmural flux of oxygen from blood to tissue increased markedly at the level of minute 4°A–5°A arterioles. At the arterial end of cortical capillaries, PO_2 averaged 57.9±10.6 mmHg, n=19, (or, in terms of blood oxygen saturation SO_2, 82±9%) and 258±19 µm downstream 40.9±11.5 mmHg, n=19, (SO_2 59±18%). The averaged PO_2 drop on the capillaries studied was 17±9 mmHg, and the longitudinal PO_2 gradient was accordingly 0.07±0.04 mmHg/µm (SO_2 0.1±0.06%/µm). The radial profiles of tissue PO_2 recorded near arterioles, capillaries and venules clearly demonstrated that all these microvessels supply oxygen to brain tissue. The PO_2 distribution on venules was characterized by pronounced heterogeneity.

Key words Oxygen tension · Arterioles · Capillaries · Venules · PO_2 gradient · Brain cortex · PO_2 microelectrode · Oxygen diffusion

Introduction

Oxygen tension (PO_2) in blood of brain microvessels is one of the basic parameters of cortical oxygenation, as it determines the PO_2 gradient and thus, according to Fick's law, the oxygen flux from microvasculature to respiring nerve cells. The wall of cortical arterioles and venules is permeable to oxygen [5, 6, 9–11]. These microvessels therefore, in conjunction with capillaries, participate in gas exchange between blood and tissue.

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Although several authors have reported values for the oxygen tension (or degree of blood saturation with oxygen, SO_2) in pial arterioles [6, 11, 21], or in pial venules [21, 22], or in cortex capillaries [11, 25], the distribution of PO_2 throughout the microvascular tree of the brain cortex still remains poorly investigated. The lack of quantitative data makes an analysis of the mechanisms of oxygen supply to the nerve cells largely incomplete.

A methodical approach that has been developed in our laboratory combines the techniques of intravital microscopy (using contact lens epiobjectives) and PO_2 measurements by needle oxygen microelectrodes. This approach makes it possible to conduct PO_2 measurements on different sites of cortical microvasculature (on the surface of arterioles, capillaries, venules), or in tissue at various distances from the microvessel. Thus, this method allows correlation between tissue PO_2 values and the geometrical parameters of cortical microvasculature.

The main purpose of the present work was to study systematically the distribution of PO_2 in microvasculature (arterioles, capillaries and venules) of rat brain cortex in longitudinal and radial directions to the vessel wall. Our study is the first to measure PO_2 directly at two points along the length of the same capillary (at the arterial end and 258±95 µm downstream).

Materials and methods

The experiments were performed on male Wistar rats weighing 220–260 g. Animals were anaesthetized with pentobarbital sodium (dose for surgery 50 mg/kg i.p., supplementary doses 15–20 mg/kg per h, s.c.). Teflon catheters (0.8 mm OD) filled with heparin-saline were inserted into the femoral artery and vein for monitoring mean arterial blood pressure, for blood sampling and for compensatory infusions of blood from a donor rat. The pH, PCO_2 and PO_2 in blood samples were measured with a blood gas analyser BME-3 (Radiometer, Denmark). The head of the animal was mounted in a frame, and the skull was exposed by a midline excision. Trepanation (diameter 8 mm) was performed using a saline-cooled dental drill. The dura mater was removed carefully and the pial surface superfused with a solution of the following composi-

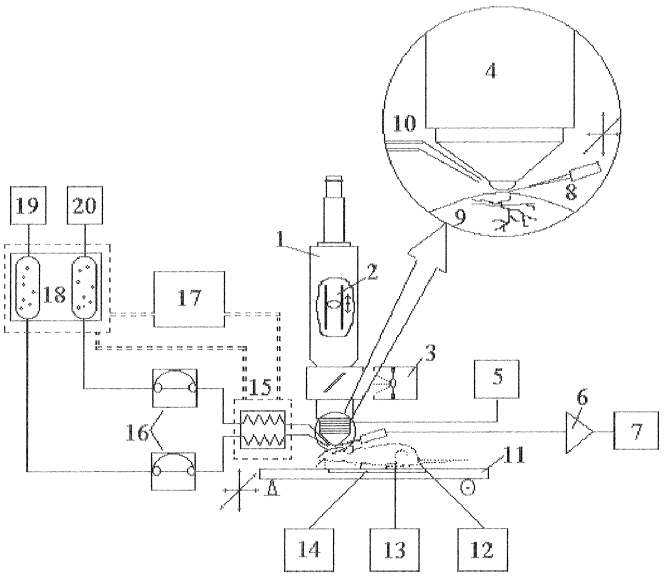

Fig. 1 Schematic diagram of the experimental system. *1* microscope Lumam-K1 with contact lens epiobjective; *2* focusing lens; *3* xenon lamp; *4* contact lens epiobjective; *5* objective thermostabilization system; *6* electrometric amplifier; *7* line chart recorder; *8* PO_2 microelectrode attached to 3D-micromanipulator; *9* brain cortex tissue; *10* tubing carrying the calibration and superfusion solutions to epiobjective lens; *11* platform for the animal with heating pad and 3D-micromanipulator system; *12* core temperature recording; *13* blood arterial pressure monitoring system; *14* heating pad; *15* heat exchangers; *16* peristaltic mini pumps; *17* thermostat U-10; *18* saturator; *19* air microcompressor; *20* cylinder with gas mixture 5% O_2, 5% CO_2, 90% nitrogen

tion (in mM): NaCl 118, KCl 4.5, $CaCl_2$ 2.5, KH_2PO_4 1.0, $MgSO_4$ 1.0, $NaHCO_3$ 25, glucose 6. The solution was saturated with a gas mixture of 5% O_2, 5% CO_2, nitrogen remainder, and had a pH of 7.35±0.08 at 37±1 °C. During experiments animals breathed spontaneously with room air. Oxygen dissociation curves (ODC) of the arterial blood samples were analysed using the HEM-O_2-SCAN (Aminco, USA). As ODCs were constructed under standard conditions (a PCO_2 of 40 mmHg), they were recalculated for the in vivo conditions of arterial and venous blood.

The schematic illustration of the experimental installation is shown in Fig. 1. The base of the microscope (1) and platform for the animal (11) were fixed to the wall to prevent any mechanical displacements during PO_2 measurements on minute microvessels. The animal was placed on the platform (11) its head was fixed in a special frame using a teeth-fixing rod. The platform could be handled with a precise 3D-manipulator. The temperature of the frontal lens of the epiobjective (4) was maintained at 36–37 °C. The core temperature of the animal was maintained at 37±1 °C with the heating pad (14).

Visualization

The brain cortex microvessels were visualized using a special biological microscope Lumam-K1 (Lomo, Russia) supplied with contact lens epiobjectives (×10/0.30, ×20/0.60). In this work an objective ×10 and ocular lens ×12, ×15, or ×25A were used. The diameter of microvessels was estimated using an ocular micrometer with an accuracy of 3–5 μm; the length of capillaries with an accuracy of 10–20 μm. The maximal depth of vision was about 120–150 μm. The focus was changed by means of a special lens (2), positioned along the optical axis of the microscope draw-tube without any displacement of the objective relative to the tissue surface (9) (Fig. 1). During PO_2 measurements the frontal lens (diameter approximately 5000 μm) was in contact with tissue being studied (no compression, the gap between lens and tissue surfaces was less than 10 μm). This small gap prevents convection of the superfusion solution over the studied microregion of brain tissue. The superfusion solution was saturated with 5% CO_2 (to exclude elimination of CO_2 from blood of microvessels) and 5% O_2 (to minimize the PO_2 gradient between solution and cortical tissue). We assume therefore that the influence of superfusion solution on PO_2 measurements in tissue was low, or absent.

PO_2 microelectrode

PO_2 was measured using platinum microelectrodes, recessed type, (recess length 2–4 μm), glass insulated, with tip diameters including isolation of 3–5 μm. The microelectrodes were manufactured by the author. The platinum wire (diameter 30 μm) was sharpened electrochemically to a tip diameter of about 1.0 μm. A piece of sharpened wire, length 10–12 mm, was welded to the copper wire (diameter about 100 μm) in the fire of miniature burner. Then the platinum tip was inserted into a glass capillary micropipette and was welded to the glass using an electrically heated loop. The tip of the microelectrode was bevelled, and the recess was electrochemically made. The electrode was cleaned in boiling distilled water, then the tip covered by a collodion membrane permeable to oxygen. The oxygen sensitivity of the microelectrodes was 2–6 pA/mmHg PO_2 in physiological solution saturated with atmospheric air. The electrodes, with negligible residual current in "zero solution" (saline with PO_2 less than 3–5 mmHg) and relatively stable characteristics of oxygen sensitivity (drift less than 50%/h), were used in experiments. The electrodes were insensitive to stirring: convective mixing of solution with linear velocity of 30–50 mm/s resulted in an increase of polarographic current of less than 3% of its initial value. They were also practically insensitive to differences in the O_2 diffusion coefficient between tissue and the calibration solution.

During experiments the microelectrodes were calibrated in air-saturated superfusion solution. Stability of pH was provided by appropriate changes in NaCl/$NaHCO_3$ concentrations. Superfusion and calibration solutions were transported alternatively in separate glass tubes (rate of pumping was about 5–8 ml/min) to the objective lens, where the microelectrode tip was located (Fig. 1). This ensured an easily repeated calibration procedure immediately before and after a series of measurements. Repetition of the procedure was determined by the rate of drift of the electrode. To minimize the calibration error the tip was inserted in brain tissue or placed at the wall of large pial arteriole for several minutes to stabilize its current. Then the electrode was placed into calibration fluid again. This procedure was repeated until a stable level of the electrode sensitivity to oxygen was attained. During experiments the additional control of calibration was performed by measuring PO_2 values at the surface of large (50–70 μm) pial arterioles and comparing the obtained values with the PO_2 measurements in systemic arterial blood.

PO_2 measurements on microvessels

All PO_2 measurements on microvessels were intended to assess the intravascular PO_2. This is not a problem for capillaries and the smallest thin-walled arterioles and venules. When measuring PO_2 at small arterioles (calibre less than 25–30 μm) the tip of electrode was impressed slightly into the wall. During PO_2 measurements at capillaries the tip was placed close to the capillary wall. Special attention was paid so that the blood flow in the studied and adjacent capillaries was not disturbed by manipulations with the microelectrode (visual assessment). A transmural PO_2 gradient exists across the wall of large arterioles [6]. Duling et al. (1979) have shown that the PO_2 gradient averages 0.9 mmHg/μm distance. In large arterioles of the rat (luminal diameter 40–70 μm, the wall thickness is about 8–15 μm) the radial PO_2 gradient may lower PO_2 values recorded on the outer surface of the wall substantially.

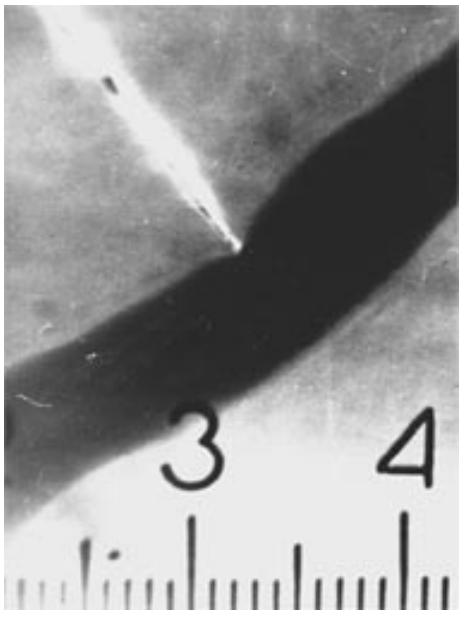

Fig. 2 Oxygen microelectrode positioned on the surface of a rat pial arteriole (30 μm diameter). *Small division* of *scale* 5 μm

To minimize the measurement error the fine tip of PO_2 microelectrode was therefore impressed into the wall parenchyma so that the distance from the blood stream was less than 3–4 μm (Fig. 2). We assume that the PO_2 electrodes used in these experiments obtain their readings from the lumen (capillaries, small microvessels), or from inner surface of the wall (large arterioles). In every case the measurement error is within several millimetres of Hg. It may be greater for large arterioles under conditions of hyperoxia or hypoxia, when the radial PO_2 gradient in the wall may be increased greatly.

Intravascular PO_2 measurements were performed in some large (diameter of 100 μm or more) venules. Insertion of the tip into blood stream generally resulted in instantaneous thrombus formation (on the tip). Procedures were developed, however, to minimize the influence of thrombus formation on PO_2 measurements in venular blood.

Radial profiles of tissue PO_2

The PO_2 microelectrode was positioned perpendicular to the wall of selected microvessel. The PO_2 was measured at the wall surface, as described above and at distances of 10, 20, 30 μm and so on from the vessel wall, sequentially. Then the electrode was returned to the wall to re-check the value obtained. It should be stressed here, that as superficial microvasculature forms a tortuous thick network, it was not easy to select a site for recording a radial PO_2 profile from a given microvessel. Therefore, the PO_2 microelectrode was positioned so that the nearest large microvessels (on the course of the electrode track) was at least 80–100 μm distant.

Protocol

At least 15–20 min was allowed for the tissue to stabilize, prior to the acquisition of PO_2 data. Measurements of PO_2 on pial and cortical arterioles and venules were performed in every experiment. One can not be certain however, that the studied microvessels were contiguous as the optical systems restricted the depth of focus to about 120–150 μm from the brain surface.

The PO_2 was measured on the surface of a large 1°A (first-order branch) order arteriole and an arterial blood sample taken simultaneously. (These paired measurements were repeated several times during the experiment and were used as an additional control of microelectrode sensitivity). Then multiple PO_2 measurements were performed on arteriolar and venular microvessels and radial PO_2 gradients were recorded as described above.

The PO_2 drop along the capillary length was assessed by sequential measurements of PO_2 at two points on the same capillary. The time interval between these two measurements was 20–30 s.

On completion of the experiment a blood sample (approximately 200 μl) was taken from the superior sagittal sinus using a sharpened (tip diameter about 0.7 mm OD) glass capillary.

All data are presented as mean±SD. The standard Student's *t*-test was used to compare the means of the PO_2 values at microvessels of various branching orders. $P<0.05$ was accepted as significant.

Results

The data presented were collected from 26 animals. The average arterial pressure of studied animals was in the range of 90–120 mmHg and did not vary significantly during the experiments. The systemic arterial blood gas values were: pH 7.37±0.05, PCO_2 34.2±7.0 mmHg, PO_2 85.6±10.1 mmHg (n=40) and P_{50} 36±2 mmHg, n=9 (where P_{50} is the blood PO_2 at which haemoglobin is 50% saturated). The oxygen saturation (SO_2) values were calculated using the ODC and measured PO_2 values; for systemic arterial blood SO_2 averaged 95±6%. The samples of blood taken from the confluence of sagittal sinuses had the following characteristics: pH 7.36±0.08, PCO_2 40.5±5.7 mmHg, PO_2 43.5±5.2 mmHg and SO_2 62±7%, n=28.

Arterioles

All arterioles studied were grouped according to their order of branching. The branches of the middle cerebral artery were designated first-order arterioles (1°A), their daughter branches as second-order arterioles (2°A), etc. The main results of PO_2 measurements on arterioles are presented in Table 1. The data indicate that the mean PO_2 in 1°A arterioles is lower than in systemic arterial blood (P=0.014). We assume that the decrease in PO_2 is attributable to the presence of an arteriolar wall transmural PO_2 gradient and/or a real decline of PO_2 in the blood of 1°A arterioles. The average PO_2 values in 2°A–5°A arterioles were significantly lower than in systemic arterial blood ($P<0.001$). The data therefore show a significant fall in PO_2 along the pial microvasculature: from 81.2±6.2 mmHg (or 94±2%SO_2) on 1°A arterioles to 61.5±12 mmHg (83±11%SO_2) on 5°A arterioles. The decrease of oxygen saturation in arteriolar blood (ΔSO_2) relative to systemic arterial blood was also estimated. This value may be considered as a measure of diffusional losses of oxygen from the proximal (relative to a given arteriole) portion of the cortical microvasculature. It clearly indicates that the transmural diffusional flux of oxygen increases markedly at the level of minute (4°A–5°A) arterioles (Table 1).

The radial tissue PO_2 profiles near cortical arterioles (Fig. 3a) indicate how the tissue PO_2 values correlate

Table 1 Distribution of oxygen tension (PO_2) on cortical arterioles of the rat brain cortex. Values are mean±SD for *n* measurements (*D* arteriolar diameter, SO_2 saturation of blood haemoglobin with oxygen, ΔSO_2 difference in SO_2 between systemic arterial and arteriolar blood)

Branching order	*D* (μm)	PO_2 (mmHg)	SO_2 (%)	ΔSO_2 (%)
1°A	45±7, *n*=42	81.2±6.2*, *n*=48	94±2	1
2°A	33±7, *n*=53	78.7±8.9**, *n*=55	94±5	1
3°A	26±7, *n*=38	75.8±11.1**, *n*=43	93±6	2
4°A	13±4, *n*=38	68.3±8.3**, *n*=35	89±8	6
5°A	8±2, *n*=40	61.5±12**, *n*=40	84±11	11

*, **P<0.05, <0.001 respectively vs systemic arterial PO_2

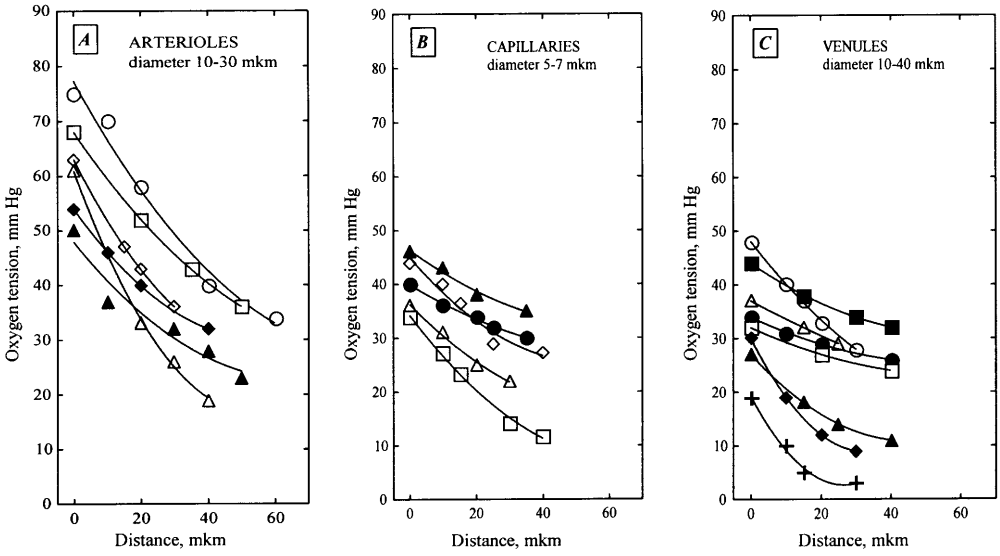

Fig. 3 The radial profiles of tissue PO_2 at precortical arterioles (**A**), capillaries (**B**) and cortical venules (**C**) of the rat. *X-axis* distance in micrometer; *Y-axis* PO_2 in millimetres of Hg. Each *symbol* represents an individual value. The same symbols in **A**–**C** are independent of one another

Table 2 PO_2 on arterial and venous ends of cortical capillaries of the rat during normoxia

No.	Length of capillary segments (μm)	PO_2 (mmHg)		ΔPO_2 along capillary length (mmHg)
		arterial side	venous side	
1	130	47	20	27
2	250	52	46	5
3	450	64	47[a]	17
4	210	45	38	7
5	360	35	20	15
6	270	69	47[a]	22
7	300	52	24	28
8	210	59	42	17
9	450	47	33	14
10	300	52	33	19
11	100	55	51	4
12	280	54	50	4
13	160	60	45	15
14	260	66	42	24
15	150	65	58[a]	7
16	270	66	35	31
17	260	66	51	15
18	300	80	46	34
19	200	66	53	13
Mean:	258	57.9	40.9	17.0
±SD:	95	10.6	11.1	9.1

[a] PO_2 recorded at the actual venous end (optically confirmed) of the capillary (see text for details)

with position along the vascular tree. These data show that the highest PO_2 values in brain tissue are related to the cortical arterioles, and not to the arterial end of the capillary, as has been assumed.

Capillaries

A total 19 capillaries were studied (Table 2). Only capillaries 3,6,15 could be visualized along their whole length from the arterial to the venous end. In other cases the second point along the capillary length was chosen as the

maximum distance from the arterial end within the limits of vision of the optical system (the maximal depth of focus was 120–150 µm from the brain surface). The mean length of these capillary segments was about 258±95 µm, n=19). The data presented in Table 2 are the first direct measurements on brain cortex capillaries, where the PO_2 values are paired with the length of capillary segments. The data show that at the arterial end of the capillaries oxygen tension averaged 57.9±10.6 mmHg (or 82±9%SO_2), that is substantially lower than in systemic arterial blood. On the venous side of studied capillaries the PO_2 averaged 40.9±11.5 mmHg, n=19 (or 59±18%SO_2). The mean PO_2 drop between these two points was 17±9.1 mmHg, (23±14%SO_2), and, consequently, the longitudinal PO_2 gradient was 0.07±0.04 mmHg/µm (SO_2 0.1±0.06%/µm). The values for the PO_2 drop along the capillaries (ΔPO_2) and the length of the studied capillary segments (L) did not correlate significantly (r=0.21).

Venules

The most characteristic feature of the PO_2 distribution of pial and cortical venules was their pronounced heterogeneity. The individual PO_2 values varied in the range of 15–60 mmHg on venules of the same branching order. The scatter of the PO_2 values at different sites relative to the venular lumen in vessels of 100 µm in diameter and above often exceeded 10–20 mmHg (Fig. 4). A distinct difference in colour between separate streams of blood, the result of confluence of flows from small proximal venules, could be seen with the microscope. The difference in colour persisted along the length of the venular tree, including the 1°V venules.

The results of PO_2 measurements on 1°V–5°V venules are presented in Table 3. PO_2 on 5°V cortical venules averaged 38.2±12.3 mmHg (54±18%SO_2, n=59), i.e. lower than in the blood of superior sagittal sinus (P=0.032). For other groups of venules the mean PO_2 values did not differ significantly from PO_2 in the sagittal sinus (P>0.05). Also there was no significant difference in PO_2 between the groups of venules studied (P>0.05).

It was found in the present work, that all studied venules released oxygen to the tissue under normoxic conditions. The measurements of radial profiles of tissue PO_2 near the venules clearly support this finding (Fig. 3c). The data show that cortical venules of calibre 10–40 µm with a high intravascular PO_2 may supply oxygen to a tissue zone of at least 40–60 µm in diameter. It should be noted here that these PO_2 profiles were recorded in tissue regions in which large microvessels were distant from the studied venule (see above).

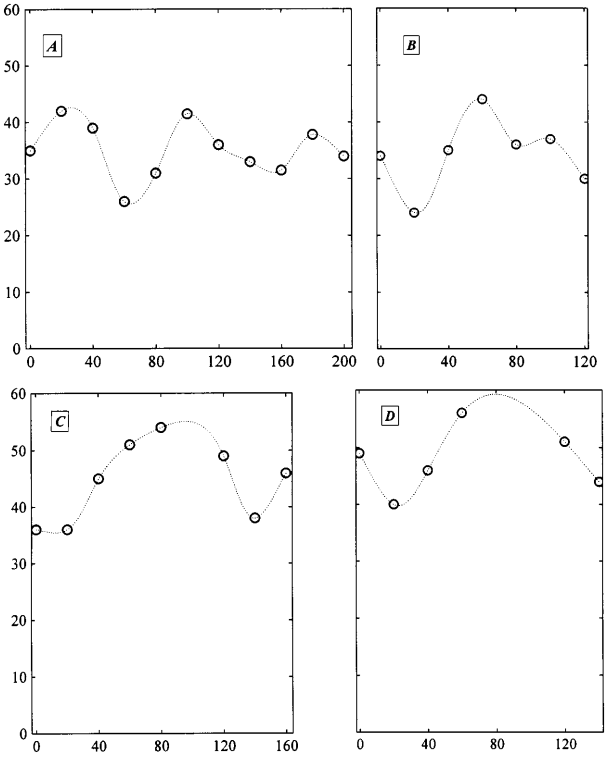

Fig. 4A–D The intraluminal profiles of PO_2 in pial venules of the rat. **A**–**D** Pial venules of 200, 120, 160 and 140 µm, respectively. *X-axis* distance in micrometres; *Y-axis* PO_2 in millimetres of Hg

Table 3 Distribution of PO_2 on cortical venules of the rat in normoxia. Values are mean±SD

Branching order	D (µm)	PO_2 (mmHg)	SO_2(%)
1°V	258±31, n=24	41.3±9.7, n=147	59±14
2°V	145±15, n=35	39.6±10.1, n=127	57±16
3°V	71±24, n=47	40.1±9.1, n=58	57±14
4°V	31±9, n=72	41.1±10.9, n=98	59±17
5°V	13±6, n=59	38.2±12.3*, n=59	54±18

*P<0.05 vs sagittal sinus blood

Discussion

The first limitation of the method used is the small depth of focus of the optical system. Care must therefore be exercised in extrapolating the results obtained in this thin tissue layer to the brain tissue as the whole. The second limitation is the accuracy of the PO_2 measurements. It is well known that platinum oxygen microelectrodes often show a marked drift in their sensitivity. By decreasing the diameter of the electrode tip, by a making recess and covering the active surface with a collodion film we reduced the calibration drift significantly. For the experiments we selected only relatively stable electrodes that were tested in vitro. During the experiments, the electrodes were calibrated before and after each series of measurements. These steps made it possible to reduce

the measurement error of the absolute PO_2 values to less than 10%. The error of relative PO_2 values (e.g. in different locations on the same capillary) was estimated as being less than 2%. Despite these limitations, the method of oxygen micropolarography and intravital contact microscopy enable correlation studies of tissue PO_2 values and microvascular geometry to be performed on such tissues as brain, liver, skeletal muscle etc.

It is known that pentobarbital anaesthesia noticeably decreases oxygen consumption and blood flow in brain tissue [15, 17], and so this might significantly affect the PO_2 distribution patterns throughout the microvasculature and brain tissue. Simultaneous measurements of local blood flow and local PO_2 distribution in the same brain areas show, however, that the PO_2 distribution in pentobarbital anaesthetized cats relative to control animals remains the same [4]. This might be accounted for by the fact that blood flow and O_2 uptake fall by approximately the same extent in the anaesthetized brain [4, 17]. Pentobarbital anaesthesia does, however, decrease regional metabolic, and hence SO_2, heterogeneity in cerebral microvasculature [17].

The data presented in this study reveal a significant fall in the PO_2 values on 1°A–5°A arterioles. The decline was about 20 mmHg, or about 10%SO_2. Similar findings have been reported by Duling and co-workers [6]. They found that the PO_2 on cat pial arterioles of calibre 22–200 μm fell from 98.8±10.7 mmHg to 72.6±3.6 mmHg. It is worth noting here, that the data available in the literature on direct PO_2 (or SO_2) measurements in brain cortex microvasculature are generally sparse and have been recorded only from microvessels of a certain calibre (30–50 μm) [8, 19, 21–23, 25]. The present study is the first to examine systematically the PO_2 distribution throughout the cerebral microvascular network of the rat.

Direct measurements of PO_2 at brain cortex capillaries are of great physiological importance, as the capillaries deliver a large portion of oxygen to the brain tissue. In our study, PO_2 was measured at two points along the length of individual capillaries: at the arterial end (PO_2 57.9±11.1 mmHg) and 258±95 μm downstream (PO_2 40.9±11.5 mmHg, n=19). No significant difference was found between the PO_2 values on 5°A arterioles and the arterial end of capillaries (P>0.05). Also there was no significant difference between the PO_2 values for 5°V venules and the venous side of the capillaries studied (P>0.05). The mean length of the capillary segments was close to the known data in literature [1, 2, 8]. We speculate therefore, that the studied capillary segments represent the major portion of the length of brain cortex capillaries; and, consequently, that the measured PO_2 drop may be considered as a lower estimate for the PO_2 difference along the length of cortical capillaries of the rat.

According to the data presented here, SO_2 values decreased by 10% on 1°A–5°A arterioles, but by 23% in capillaries. The cortical capillaries thus unload approximately twice as much oxygen to brain tissue as the arterioles. The most pronounced decrease in SO_2 in arteriolar blood (compared with systemic SO_2) occurs between 3°–5° A arterioles: 9% (Table 1). Therefore, the smallest (3°A–5°A) arterioles and capillaries, one may conclude, provide the major quantity of oxygen to brain cortex. These estimates are applicable to the rat brain cortex in normoxia. For other species, tissues and conditions these conclusions may not apply.

Our data showing a significant heterogeneity of PO_2 on the venous side of the cortical microcirculation is in agreement with the literature [17, 22, 23]. The coefficient of variation for 4°V and 5°V venules averaged 26.5% and 31.1%, whereas for 4°A and 5°A arterioles these coefficients were 12.2 and 19.5%, respectively (Tables 1, 3). In the blood of 3°V–1°V venules adjacent streams could often be seen: the result of confluence of flows from small venules that differed in colour and correspondingly in oxygenation [12]. A high PO_2 – 45–60 mmHg – was found in some venules (especially those draining blood from deeper brain structures). There are three possible reasons for this phenomenon: shunting of blood through the anatomical anastomoses, functional shunting of oxygen and tissue diffusional shunting of oxygen. There is no convincing evidence in the literature for the existence of arteriolar-venular anastomoses in brain tissue [13, 14]. It is also not clear to what extent high PO_2 values might be attributed to pentobarbital anaesthesia. If this were the case, some microvessels with higher flow may contribute more oxygen to the venous outflow than microvessels with lower flow. However, there are no favourable conditions for O_2 diffusional shunting in the brain microcirculation [3, 7]: cortical arterioles and venules do not form parallel pairs with countercurrent flows as in skeletal muscle. However, only pial arterioles and venules that cross are in immediate contact. The surface area of this contact is obviously negligible compared with the total surface area of the vessels that is available for exchange. Therefore, diffusional shunting of oxygen is probably of no significance in the brain cortex during normoxia. In hyperoxia however this is not the case: O_2 diffusional shunting does exist and may have a noticeable effect on the oxygen transport in brain tissue [18, 20].

On the basis of the above data we speculate, that large pial 1°V–2°V venules are most probably of no importance in gas exchange between blood and tissue. Small venules however may transfer a significant quantity of oxygen, as they characterized by a large surface area, comparable with that of capillaries [24]. The total oxygen flux from cortical venules to tissue cannot be assessed from this study. Nevertheless, the role of venules in oxygen supply to brain tissue is clearly important, at least in the superficial cortical layer with a large venular density, and demonstrates a need for additional experimental and modelling analyses.

The data presented in this study therefore show that all cortical microvessels (arterioles, capillaries and venules) supply oxygen to the tissue. The anatomical capillaries, nevertheless, are of major importance. Haemoglobin is unloaded there and SO_2 falls from 82±9 to 59±18%, i.e. by 23±14%. In 1°A–5°A arterioles blood is

desaturated by approximately 10%SO_2 and a large portion of this oxygen diffuses across the walls of small (7–25 µm in diameter) arterioles. We may conclude therefore that the bulk of oxygen delivery to brain tissue occurs at the level of microvessels of calibre of 20–25 µm and less.

In summary, the distribution of PO_2 along arterioles, capillaries and venules of rat brain cortex has been presented for the first time. In cortex capillaries, PO_2 has been measured at the arterial and venous ends of the same capillary. The values of mean longitudinal PO_2 gradient in the brain cortex capillaries were assessed. Also the character of PO_2 distribution on the venous side of brain cortex microvessels from post-capillary venules to the sagittal sinus has been presented for the first time.

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References

1. Auen EL, Bourke RS, Barron KD, San Filippo BD, Waldman JB (1979) Alterations in cat cerebrocortical capillary morphometrical parameters following K^+-induced cerebrocortical swelling. Acta Neuropathol (Berl) 47:175–181
2. Blinkov SM, Gleser II (1964) A brain of the man in figures and tables (in Russian). Medicine, Leningrad
3. Brodersen P, Sjersen P, Lassen NA (1973) Diffusion bypass of xenon in brain circulation. Circ Res 32:363–369
4. Demchenko IT (1983) Blood supply of awake brain (in Russian). Nauka, Leningrad
5. Duling BR, Berne RM (1970) Longitudinal gradients in periarteriolar oxygen tension. Circ Res 27:669–678
6. Duling BR, Kushinsky W, Wahl M (1979) Measurements of the perivascular PO_2 in the vicinity of the pial vessels of the cat. Pflügers Arch 383:29–34
7. Grieb P, Forster RE, Goodwin CW, Pape PC (1985) O_2 exchange between blood and brain tissue studied with $^{(18)}O_2$ indicator-dilution technique. J Appl Physiol 58:1929–1941
8. Hudetz AG (1997) Blood flow in the cerebral capillary network: a review emphasizing observations with intravital microscopy. Microcirculation 4:233–252
9. Intaglietta M, Johnson PC, Winslow RM (1996) Microvascular and tissue oxygen distribution. Cardiovasc Res 32:632–643
10. Ivanov KP, Derry AN, Samoilov MO, Semionov DG (1979) Diffusion of oxygen from minute arteries of brain cortex (in Russian). Dokl Akad Nauk SSSR 244:1509–1511
11. Ivanov KP, Derry AN, Vovenko EP, Samoilov MO, Semionov DG (1982) Direct measurements of oxygen tension at the surface of arterioles, capillaries and venules of the cerebral cortex. Pflügers Arch 393:118–120
12. Ivanov KP, Levkovich YI, Vovenko EP, Maltzev NI (1990) Separate flows of erythrocytes with different degrees of oxygenation in the venous vessels (in Russian). Fiziol Zh SSSR 76:338–344
13. Lopez-Majano V, Rhodes BA, Wagner HN Jr (1970) Arteriovenous anastomosis. Cor Vasa 12:253–274
14. Mooradian AD, McCuskey RS (1992) In vivo microscopic studies of age-related changes in the structure and the reactivity of cerebral microvessels. Mech Ageing Dev 64:247–254
15. Otsuka T, Wei L, Acuff VR, Shimizu A, Pettingrew KD, Patlak CS, Fenstermacher JD (1991) Variation in local cerebral blood flow response to high-dose pentobarbital sodium in the rat. Am J Physiol 261:H110–H120
16. Siesjö BK (1978) Brain energy metabolism. Wiley, Chichester
17. Sinha AK, Chi OZ, Weiss HR (1992) Effect of pentobarbital on cerebral regional venous O_2 saturation heterogeneity. Brain Res 591:146–150
18. Sokolova IB (1993) The role of the cerebral cortex venules in normal oxygen transport and in normobaric hyperoxia (in Russian). Fiziol Zh SSSR 79:60–65
19. Torres Filho IP, Intaglietta M (1993) Microvessel PO_2 measurements by phosphorescence decay method. Am J Physiol 265:H1434–H1438
20. Vovenko EP, Sokolova IB (1992) The distribution of oxygen pressure in the pial arterioles of the rat under normobaric hyperoxia (in Russian). Fiziol Zh SSSR 78:65–72
21. Watanabe M, Harada N, Kosaka H, Shiga T (1994) Intravital microreflectometry of individual pial vessels and capillary region of rat. J Cereb Blood Flow Metab 14:75–84
22. Wei HM, Wei YC, Sinha AK, Weiss HR (1993) Effect of cervical sympathectomy and hypoxia on the heterogeneity of O_2 saturation of small cerebrocortical veins. J Cereb Blood Flow Metab 13 :269–275
23. Weiss HR, Buchweitz E, Sinha AK (1983) Effect of hypoxic-hypocapnia on cerebral regional oxygen consumption and supply. Microvasc Res 25:194–204
24. Wiedeman MP (1963) Dimensions of blood vessels from distributing artery to collecting vein. Circ Res 12:375–378
25. Wilson DF, Pastuszko A, DiGiacomo JE, Pawlowski M, Schneiderman R, Deliviria-Papandopoulos M (1991) Effect of hyperventilation on oxygenation of the brain cortex of newborn piglets. J Appl Physiol 70:2691–2696