Capillary Perfusion of the Rat Brain Cortex
An In Vivo Confocal Microscopy Study

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Abstract Confocal laser-scanning microscopy was used to visualize subsurface cerebral microvessels labeled with intravascular fluorescein dye in a closed cranial window model of the anesthetized rat. In noninvasive optical sections up to 250 μm beneath the brain surface, plasma perfusion and blood cell perfusion of individual capillaries were studied. Under resting conditions, in all cerebral capillaries the presence of plasma flow was demonstrated by the appearance of an intravenously injected fluorescein tracer within 20 seconds after injection. Plasma flow was verified even in capillaries that contained stationary erythrocytes or leukocytes; 91.1% of the capillaries contained flowing blood cells, 5.2% contained stationary blood cells, and no blood cells were seen in 3.6%. Mean blood cell velocity was 498.3±443.9 μm/s, and the mean blood cell supply rate was 35.75±28.01 cells per second. When capillaries were continuously observed for 1 minute, “on” and “off” periods of blood cell flow were noted. During hypercapnia (increase of PCO₂ from 33.25 to 50.26 mm Hg), mean blood cell flux increased from 38.6±17.2 to 55.5±12.2 per second (P<0.05, paired t test of mean values in six animals), and blood cell velocity increased from 519.5±254.8 to 828.5±460.8 μm/s (P=0.074, paired t test of mean values in six animals). Homogeneity of blood cell flux increased as indicated by the coefficient of variation decreasing from 44.6% to 22.0%, and the portion of poorly perfused capillaries (blood cell flux, <40 per second) decreased from 59.2% to 22.4%. Capillary diameter increased from 5.33±0.25 to 5.66±0.29 μm (P<0.05, paired t test of means in five animals). Our results suggest that opening and closing of capillaries (capillary recruitment) in the classic “all or none” fashion is not a means of regulating cerebral blood flow. Rather, we suggest that alterations in heterogeneity of capillary perfusion accompanied by variations of the fraction of low-flow capillaries and capillary diameter may be important adaptatory mechanisms. (Circ Res. 1994;75:55-62.)

Key Words • capillary recruitment • cerebral blood flow • cerebral microcirculation • functional neuroimaging

It has been suggested that capillary blood flow in the brain is regulated by opening and closing of capillaries.1-3 However, this capillary recruitment hypothesis (first proposed for frog muscle by Krogh4 in 1919) has been the subject of an ongoing controversy. Studies comparing the perfused and the total brain capillary bed in histological sections or assessing the issue by measuring local cerebral blood flow and local cerebral blood volume5-11 and indirect in vivo studies12-15 have yielded conflicting findings. The feasibility of direct microscopic observation of cerebral capillaries in vivo was demonstrated in 1963 by Rosenblum and Zweifach16; however, previous studies were hamp ered by the poor depth penetration of conventional light microscopy. Therefore, those studies had drawbacks such as being limited to the most superficial capillaries without precise depth information16-18 and damage to the brain tissue caused by the use of a transilluminator.19

In the present study, we took advantage of the improved depth penetration and discrimination properties of confocal light microscopy to investigate brain cortex capillary perfusion in vivo. In a closed cranial window preparation of the rat, this new experimental approach20,21 makes the outer 250 μm of the rat brain microcirculation accessible for direct microscopic observation in vivo. We have recently shown that this method permits the analysis of morphological aspects of the microcirculation in three dimensions.22 In the present study, we adapted this method to assess hemodynamic aspects of the cerebral microcirculation. The purpose was to elucidate mechanisms of capillary blood flow regulation, with particular emphasis on the assessment of the capillary recruitment hypothesis. Specifically, the following issues were addressed: the congruence of the total and plasma-perfused capillary bed; the congruence of the total and blood-cell-perfused capillary bed; quantitative blood cell flow (blood cell flux and velocity) in individual capillaries; and changes in quantitative blood cell flow in capillaries and capillary diameter during hypercapnia.

Materials and Methods

Animal Preparation, Monitoring, and Exclusion Criteria

Forty-seven male Wistar rats (250 to 350 g) were anesthetized with thiobutabarbital (Inactin, BYK Pharmaceuticals; initial dose, 100 mg/kg body wt IP) and tracheotomized, and the left femoral artery and vein were cannulated. A continuous intravenous saline infusion was started, and the animals were artificially ventilated (Harvard rodent respirator, Harvard Apparatus). The animals were placed in a stereotactic frame. A craniotomy was performed, and the dura was incised (except in seven animals in which the dura was kept intact) and partly removed. To reestablish the integrity of the skull and ensure maintenance of normal gas tensions and extravascular pres-
sure after craniotomy, a closed cranial window (glass from a coverslip) was implanted over the right parietal bone (modifications of the method of Morii et al29). The window had two outlets, and the space beneath the window was continuously superfused with artificial cerebrospinal fluid (prepared according to the method of Levasseur et al24) at a rate of 1 mL/h. The intracranial pressure was controlled at 3±1 mm Hg by adjusting the level of the cranial window outflow catheter.

End-expiratory PCO₂ (Heyer CO₂ monitor EGM I), arterial blood pressure, and intracranial pressure (Statham P23 ID) were analog/digital-converted and recorded continuously on a PC microcomputer by use of ASYST data acquisition software (Macmillian Software). Body temperature was controlled at 38±1°C by adjusting the temperature of a heating pad, and arterial PCO₂ was controlled at 35±5 mm Hg by adjusting the respirator. Arterial blood gases (PO₂, PCO₂, and pH) were measured serially (AVL gas check model 940), and the hematocrit was measured at the beginning and at the end of each experiment.

During preparation of the animal, no cautery or hemostatics were used. Bleeding from dural vessels or erythrocytes from other sources on the brain cortex led to the exclusion of the animals (n=10). The integrity of the blood-brain barrier was continuously assessed during each experiment, and leakage of fluorescein through the blood-brain barrier at any time point during the experiment led to the exclusion of the animal (n=3).

Confocal Laser-Scanning Microscopy: Setup

We used a Bio-Rad MRC 600 confocal laser-scanning unit that was attached to a Nikon optiphot microscope. The objective was a Zeiss water immersion lens corrected for a cover slip with a numerical aperture of 0.75, a working distance of 0.16 mm, and a ×40 magnification. The light source was an Argon laser (Ion Laser Technology) with principle lines at 488 and 514 nm. For fluorescein and rhodamine fluorescence microscopy, the appropriate filter sets were used. Confocal microscopy was performed through the cranial window. The position of the confocal image plane was adjusted by moving the stage of the microscope with a stepper motor (precision, 0.1 μm). For storing dynamic sequences of images, the data were digital/analog-converted and transferred to a VHS video.

Imaging Cerebral Microvessels

The vascular system was labeled by intravenous injection of 2 mg/100 g body wt sodium fluorescein. The position of the confocal imaging plane was adjusted parallel to the brain surface. The brain surface corresponded to position 0, to which all other sections were related. Image acquisition time for a two-dimensional confocal image (768×512 matrix) was 1 second. A typical image is given in Fig 1. Based on parallel sections at different levels beneath the brain surface, three-dimensional reconstructions were performed as described by our group previously.

Dynamic Studies of Capillary Blood Cell Flow

The acquisition time for two-dimensional confocal images was too slow to adequately assess blood cell flow in individual capillaries. Therefore, a single-line (one-dimensional) imaging approach was used, sacrificing spatial resolution to gain temporal resolution. The acquisition time for a single line was 2 milliseconds. This line was placed either in the middle of a capillary parallel to the capillary walls (Fig 2) or across a capillary. By repetitive scanning with a temporal resolution of 2 milliseconds and displaying subsequent lines on the TV screen, a space-time plot of blood cell flow in individual capillaries was obtained from which blood cell flux and blood cell velocity were calculated (Fig 2).

Study I: The Congruence of Total and Plasma-Perfused Capillary Bed

Protocol a

In six animals, sodium fluorescein (2 mg/100 g body wt; the solution contained 5 mg fluorescein per milliliter of 0.9% saline solution) was injected intravenously. Twenty seconds after the injections, a confocal image was acquired through the microvascular network (253 μm×168 μm×1 μm) 50 to 60 μm beneath the brain surface. An image of the same section was again acquired 10 minutes later. To account for respiratory motion of the brain, acquisition of individual frames was triggered to respiration. The two images, representing the "perfused" (20 seconds after injection) and the "total" (10 minutes after injection) capillary bed, were subsequently compared. According to the findings of Weiss, suggesting the existence of unperfused capillaries, one would expect a significant portion (~50%) of capillaries not to be visible 20 seconds after tracer injection. Ten minutes later, however, these capillaries would become visible.

Protocol b

In eight animals, 10 to 60 minutes after intravenous injection of sodium fluorescein (2 mg/100 g body wt), optical sections (50 to 249 μm beneath the brain surface, respiration-triggered) through the capillary network were monitored continuously for 40 seconds before, during, and after the injection of additional bolus (0.2 mg in 0.1 mL of 0.9% NaCl) of fluorescein.
FIG 2. Assessment of intracapillary hemodynamics by schematics (top panels) and imaging (bottom panels). To improve temporal resolution for the assessment of intracapillary hemodynamics, spatial resolution was sacrificed by using a one-dimensional imaging approach. A single scan line was placed in the midline of a straight part of a capillary that had a straight segment of at least 25-μm length (schematic, top left; in vivo example, bottom left). This line was repetitively imaged with a temporal resolution of 2 milliseconds. Since fluorescein, which served as vascular label, is only present in the plasma, blood cells appear as nonlabeled interpositions (dark) between the labeled plasma gaps (white). Hence, in the one-dimensional image, a pattern of black and white stripes is generated (top left). When blood cells are moving, this pattern moves in the direction of blood cell flow. When subsequent lines are displayed vertically displaced on the computer screen, a space (horizontal axis)–time (vertical axis) plot is generated (schematic, top right; in vivo example, bottom right). In this plot, the velocity of individual blood cells is determined by dividing the distance (d) a certain blood cell travels by the time (t) needed. The blood cell flux is given by the number of blood cells passing per second. The bottom panels give an in vivo example. The optical section (bottom left) is 147 μm beneath the brain surface. The oblique black and white stripes in the two-dimensional images correspond to moving blood cells (black) and plasma gaps (white). The horizontal line is the scan line placed within the horizontal capillary segment. The space–time plot derived from repetitive one-dimensional imaging is given in the bottom right panel. Bars = 10 μm.

An increase of the fluorescent signal in a capillary within 20 seconds after administration of the bolus would indicate the presence of plasma flow; lack of increase would indicate the absence of plasma flow.

Protocol c

By assessing the intracapillary fluorescent enhancement after intravenous fluorescein injection, the presence of plasma flow was evaluated in 17 of 192 capillary segments (in eight animals) in which stationary or no blood cells were seen (see study 2).

Protocol d

In five animals, by assessing the intracapillary fluorescent enhancement after intravenous injection of fluorescein (0.2 mg in 0.1 mL of 0.9% NaCl), the presence of plasma flow was evaluated in capillary segments containing stationary leukocytes. Leukocytes were visualized by in vivo labeling with the intravenous administration of rhodamine 6 G (see Villringer et al38). Randomly chosen sections through the microvascular network were continuously monitored until a stationary (no visible movement for >10 seconds) leukocyte was observed. Then 0.2 mg fluorescein in 1 mL of 0.9% saline was injected intravenously, and the capillary segment was continuously monitored for the following 40 seconds.

Study 2: The Congruence of Total and Blood Cell-Perfused Capillary Bed

Ten to 60 minutes after intravenous injection of 2 mg/100 g body wt sodium fluorescein, 192 randomly chosen capillary segments (41 to 250 μm) in eight animals were each continuously monitored for 1 minute by use of the one-dimensional imaging technique described above. At the beginning of the observation period, each capillary segment was categorized into “containing flowing blood cells,” “containing stationary blood cells,” and “containing no blood cells.” During the subsequent observation period of 1 minute, interruptions of blood cell flow for >10 seconds were labeled “off periods of blood cell flow.”
Study 3: Blood Cell Flow Within Single Capillaries
To assess intracapillary hemodynamic parameters, the single-line approach described above was used. In capillaries with a straight segment of at least 25 μm, a single line (placed in the middle of the capillary parallel to the capillary walls) was scanned repetitively as described above (Fig 2). In those capillaries, mean blood cell velocity and blood cell supply rates were determined. In the remaining capillary segments (no straight segment of at least 25 μm), blood cell supply rates were measured.

To control for a potential influence of the dura incision on capillary blood cell flux and blood cell velocities, we measured those parameters also in seven animals with intact dura mater. Ninety-two capillary segments (40 to 175 μm beneath the brain surface) were randomly chosen for these experiments.

Study 4: Influence of Hypercapnia on Capillary Blood Cell Velocity, Flux, and Capillary Diameter
In six animals, blood cell velocity, flux, and capillary diameter were studied under normocapnia and during hypercapnia induced by ventilation with 5% CO2. Measurements were performed on identical capillary segments under these two conditions.

Results
Study 1: The Congruence of Total and Plasma-Perfused Capillary Bed
Protocol a
A total of 74 capillary segments were observed 20 seconds after the administration of intravenous fluorescein in six animals. When the same confocal sections were imaged again 10 minutes later, the same capillaries were seen; no capillary had appeared or disappeared.

Protocol b
In all 150 capillary segments (eight animals) that were imaged between 10 and 60 minutes after fluorescein injection, an enhancement of the intracapillary fluorescent signal was noted within 20 seconds of fluorescein administration.

Protocol c
All 17 capillary segments (of 192 capillary segments in eight rats) containing stationary blood cells or no blood cells were filled with fluorescein within 20 seconds after intravenous administration of a fluorescein bolus.

Protocol d
All 10 capillary segments (five animals) containing rhodamine-labeled stationary leukocytes were filled with fluorescein within 20 seconds after fluorescein injection.

Study 2: The Congruence of Total and Blood Cell–Perfused Capillary Bed
At the beginning of the 1-minute observation period of 192 randomly chosen capillary segments, in 175 (91.1%) moving blood cells were observed, in 10 (5.2%) stationary blood cells were visible, and in 7 (3.6%) no blood cells were observed. During the 1-minute observation period, no blood cell flow occurred for >10 seconds in 7 capillaries that initially contained flowing blood cells, and in 8 of the previously unperfused capillary segments, blood cell flow appeared.

Study 3: Blood Cell Flow Within Single Capillaries
Mean blood cell velocity was 498.3±443.9 μm/s (n=70) in animals with incised dura and 539.4±330.5 μm/s in animals with intact dura (33 capillary segments).

Mean blood cell flux was 35.75±28.01 blood cells per second in animals with incised dura mater (n=192) and 35.92±21.08 blood cells per second in animals with intact dura (n=92).

The frequency distributions of capillary blood cell velocity and flux are given in Fig 3.

Study 4: Influence of Hypercapnia on Blood Cell Velocity, Flux, and Capillary Diameter
During hypercapnia induced by ventilation of the animals with 5% CO2, arterial PCO2 increased from 33.25 to 50.26 mm Hg (n=6). Blood cell flux, blood cell velocity, and diameter of individual capillaries during normocapnia and hypercapnia were measured in 49, 20, and 51 capillaries, respectively. Mean blood cell flux (expressed as mean of the mean values obtained for each animal) increased from 38.6±17.2 to 55.5±12.2
cells per second (n=6, P<.005 by paired t test). The mean of all 49 sampled capillaries increased from 38.7±30.7 to 55.6±29.6 cells per second. The frequency distribution of blood cell flux under normocapnia and hypercapnia is given in Fig 4. Note that the number of capillaries with low perfusion (blood cell flux <40 cells per second) decreased from 59.2% to 22.4%. The coefficient of variation of blood cell flux (ratio of standard deviation to the mean) decreased from 44.6% to 22.0%. Mean blood cell velocity (expressed as the mean of the mean value for each animal) increased from 519.5±254.8 to 828.5±460.8 μm/s (P=.08 by paired t test). The mean of all 20 capillaries increased from 546.0±383.4 to 717.5±406.3 μm/s. Capillary diameter (expressed as the mean of the mean values for each animal) increased from 5.33±0.25 to 5.66±0.29 μm (P<.05 by paired t test, n=5), and the mean of all 51 capillaries increased from 5.33±0.77 to 5.68±0.81 μm. During hypercapnia, no vessel became visible that was not visible under normocapnia.

**Discussion**

In his work on muscles of frogs and guinea pigs in 1919, Krogh4 noted that under resting conditions, “most of the capillaries are in a state of contraction and closed to the passage of blood” and that a large number of capillaries opened after stimulation. Krogh himself did not observe capillary recruitment in the brain. However, others have reported findings supporting the existence of capillary recruitment.1-3,5-7,12,14,27,28 Because the results of other studies did not support this concept,5-11,15,29-31 the issue has remained controversial, and it has been further confounded because of different definitions of the term “capillary recruitment.” Krogh’s concept of capillary recruitment4 referred to both plasma and erythrocyte flow. Since erythrocyte flow must be accompanied by some plasma flow, studies that address plasma perfusion of capillaries11,28,32 implicitly use this definition. However, other authors have extended the definition to refer to erythrocyte-perfused capillaries.2,3,14,33 The presence of pure plasma flow versus corpuscular blood flow has different physiological meanings. The distinction may be of particular importance in pathophysiological settings, eg, in cerebral ischemia,34 and in the correct interpretation of new neuroimaging techniques based on the plasma distribution of an intravascular tracer.35 Therefore, we addressed plasma perfusion and blood cell perfusion and its confluence with the total capillary bed in two separate studies (studies 1 and 2). The results of these two studies indicate that the dichotomy of the capillary recruitment concept (open=perfused versus closed=not perfused) is not sufficient to describe the functional state of capillary perfusion adequately. Consequently, in studies 3 and 4 we quantitatively addressed blood cell flow in perfused capillaries by measuring the parameters blood cell velocity and blood cell flux (number of blood cells passing a capillary segment per second) under resting (study 3) and stimulated (study 4) conditions.

Our methodological approach was a new rat model for the study of the cerebral microcirculation that we have recently established using confocal laser-scanning microscopy (CLSM) in a closed cranial window preparation.20,21 In noninvasive optical sections, this method permits the observation of cerebral microvessels up to 250 μm beneath the brain surface. The temporal resolution of the method is low (on the order of 1 second); however, assessment of intracapillary hemodynamics has been possible using a one-dimensional imaging approach, sacrificing spatial for temporal resolution. Regarding the physiological validity of this method, we have shown in this and previous studies20,21 that the blood-brain barrier remains intact (see Dörnig et al33 and the present study), that the response to hypercapnia remains intact (see Villringer et al20 and the present study), and that even 1 hour of continuous illumination of a single capillary does not affect blood cell hemodynamics (we have seen intracapillary stasis only when we increased laser power 10- to 100-fold32). Further support for the integrity of the preparation in this experimental setup is the observation of very little leukocyte activation (as indicated by sticking of leukocytes to venules or extravasation) under normal conditions.26,36,37 On the other hand, we have been able to study leukocyte activation under conditions such as cerebral ischemia36,37 and bacterial meningitis36 in the same experimental setup. In the present study, we have shown that the incision of the dura mater does not measurably influence intracapillary hemodynamics. Furthermore, we have shown that CLSM laser illumination does not induce measurable temperature changes beneath the cranial window and that the local hemodynamic response to whisker stimulation remains intact (A.Them and U. Lindauer, unpublished data). Thus, although presently we cannot entirely exclude any damage induced by our experimental approach, so far, we have not found any evidence for such; rather, we...
have demonstrated that major physiological functions remained intact.

By use of this method, we have obtained the following main results: For study 1, there is complete congruence of existing and plasma-perfused capillaries. For study 2, there is an incomplete (=90%) congruence of existing and blood cell-perfused capillaries at a given time point, and in individual capillaries, on and off periods of blood cell flow occur. For study 3, there is pronounced heterogeneity of capillary blood cell flow under resting conditions with a large fraction of capillaries with only poor blood cell perfusion. For study 4, during stimulation of cerebral blood flow induced by hypercapnia, blood cell flux and blood cell velocity increase, the portion of capillaries with only little blood cell flux decreases sharply, and capillary diameters increase slightly. Subsequently, these findings are discussed in the above order.

Study 1: Congruence of Existing and Plasma-Perfused Capillaries: The Capillary Recruitment Hypothesis

Two extensive recent studies on this issue have been performed by the groups of Weiss8,9,29 and Kuschinsky.8,9,29 The experimental paradigm of these studies has been to inject an intravascular tracer and to compare the labeled (ie, perfused) capillaries with the entire counterstained capillary bed in histological sections. Weiss and colleagues found that 20 seconds after application of the intravascular tracer ~50% of the capillaries were labeled and that the total capillary network was filled after only ~6 minutes. However Kuschinsky and colleagues have reported a complete congruence of the existing and the plasma-perfused capillary bed. These contradictory findings may be due to different methods of fixing the brain sections (alcohol impregnation versus air drying) and of counterstaining the whole capillary bed (alkaline phosphatase versus fibronectin antibodies), which represent controversial methodological issues of studies in which analysis is based on histological sections.

In our experimental paradigm, we attempted an in situ analogue of those approaches: In noninvasive optical sections obtained with CLSM in vivo, we compared the perfused with the entire capillary bed. As in the studies from the groups of Weiss1,28 and Kuschinsky,8,9,29 the arrival of a fluorescent tracer in a capillary within 20 seconds after intravenous injection was used to indicate the presence of plasma flow in this capillary. As an indicator of the total capillary bed, we regarded all capillaries labeled ≥10 minutes after tracer injection. There is general agreement (even between the groups of Weiss and Kuschinsky) that after this circulation time essentially all brain capillaries are labeled. According to this assumption, the following findings of our study indicate the congruence of plasma-perfused and the entire capillary network, thus confirming fully the findings of Kuschinsky's group: (1) The capillary bed observed 20 seconds after tracer injection was identical to the one visible 10 minutes later. (2) All capillaries observed 10 minutes after intravenous fluorescein application were further enhanced within 20 seconds after application of an additional fluorescein bolus.

The following control experiments confirm these findings: (1) At a given time point, 91.1% of all capillaries contained moving blood cells and hence plasma flow. In the remaining capillaries, presence of plasma flow was verified by the appearance of fluorescein within 20 seconds after bolus injection. (2) In capillaries containing stationary leukocytes, plasma flow was present. (3) In rats with intact dura mater, the congruence of the plasma-perfused and the total capillary bed was confirmed; thus, the incision of the dura does not influence the findings of this study.

The results of the present study are supported by a recent study by Williams et al.31 These authors assessed the potential role of vascular recruitment in spatial and temporal cycling of cerebral blood flow. By comparing the distribution of two different fluorescent tracers, one after 10 and one after 60 seconds of circulation time, and by using quantitative autoradiography, these authors did not find any indication of a significant role of vascular recruitment in the rat brain cortex.

Although these and our experiments were performed under resting conditions, the congruence of the perfused and the entire capillary network implies that no capillary recruitment can take place under stimulated conditions. This is confirmed by our observations in hypercapnic animals, in which we never observed capillaries that were not visible under resting conditions. In accordance with our results, recent work by Fenstermacher's group indicates that capillary recruitment does not play a major role in adapting blood flow during hypercapnia, hypoxia, and barbiturate anesthesia. In accordance with the present study, it is suggested that the changes in blood cell velocity within previously perfused capillaries may be most important in adapting cerebral blood flow to different needs.

Since the present study was limited to the outer 250 μm of the rat brain parietal cortex, the question arises whether these findings may be applied to other regions of the brain. Since the controversy on the congruence of the plasma-perfused and the whole capillary bed refers to all examined brain structures including, explicitly, the brain cortex (Weiss and colleagues found unperfused capillaries in all parts of the brain, whereas the groups of Kuschinsky and Vetterlein found congruence of perfused and existing capillaries in all brain regions), it seems plausible to assume that the results of the present study on brain cortex capillaries may have implications for other parts of the brain as well. However, structural and functional heterogeneities of the cerebrovascular system have been reported within the brain. Hence, it is noteworthy that the recent studies by Fenstermacher's group support our conclusions for other brain regions: In most brain regions, changes in blood flow were not accompanied by similar changes in blood volume; thus, it was concluded that the major mechanism of adjusting blood flow was a change in the velocity of microvessel perfusion and not the perfusion of previously unperfused capillaries. In some brain regions, a change in microvascular plasma and blood volume was noted when blood flow was altered; however, these findings do not necessarily imply the existence of unperfused capillaries, since they may also be explained by microvascular dilatation, as we observed in the present study (see below).
Study 2: The Congruence of Total and Blood Cell–Perfused Capillary Beds

In contrast to the complete congruence of plasma-perfused and the total capillary network, we found incomplete congruence between blood cell–perfused and the total capillary network. In addition to capillaries containing moving blood cells, there were also purely plasmatic capillaries or capillaries containing stationary blood cells. Furthermore, “on” and “off” periods of blood cell flow were seen. These findings correspond to those reported by Pawlik et al.,19 most capillaries contained moving blood cells already under resting conditions, blood cell perfusion of previously unperfused capillaries does not offer a large reserve for the increase of capillary blood flow.

Hence, neither complete opening or closing of capillaries for plasma perfusion nor fluctuations of blood cell flow within capillaries seem to be a major adaptive mechanism of capillary blood flow in the brain. Therefore, instead of classifying the functional state of capillary perfusion only by an on/off dichotomy, we attempted to assess the perfusion of individual capillaries by blood cells quantitatively.

Study 3: Quantitative Assessment of Intracapillary Blood Cell Flow

Using a one-dimensional imaging approach, we were able to obtain a 2-millisecond temporal resolution, which is adequate to measure blood cell flow within single capillaries continuously. The measurements were performed in straight capillary segments with a minimum length of 25 μm. Thus, in theory, we were able to detect blood cells moving at a velocity of up to 12.5 mm/s, which is well beyond the maximal blood cell velocity that we have observed. The mean values for blood cell velocity that we obtained (498.3±443.9 μm/s) are somewhat lower than the ones reported by Ivanov et al.20 for the rat brain (790±300 μm/s). However, those experiments were performed in awake rats. It is well known that barbiturate anesthesia decreases cerebral blood flow,41 and it has been shown that red blood cell velocity is also decreased.11 We have not found values for blood cell flux in brain capillaries in the literature.

The analysis of the frequency distribution of blood cell velocities and flux (Fig 3) in the present study shows a very pronounced heterogeneity of capillary blood cell flow. This finding confirms conclusions derived from indirect in vivo studies,15,42 and it emphasis the necessity of considering this heterogeneity in order to interpret those in vivo methods correctly.42

The pronounced heterogeneity of capillary blood cell perfusion and the wide range of perfusion parameters suggest that under resting conditions many capillaries are not maximally used. If capillary recruitment is not the major adaptive mechanism of blood cell flow, one would expect that the frequency distribution of capillary perfusion parameter must change dramatically under stimulated conditions.

Study 4: Capillary Blood Cell Flux, Velocity, and Diameter Under Stimulated Conditions

By comparing the frequency distribution of blood cell flux in identical capillaries under resting conditions and hypercapnia, we were able to demonstrate that the expected pronounced change really occurred. During hypercapnia, particularly the fraction of poorly (blood cell) perfused capillaries was sharply reduced from 59.2% to 22.4%. The relative homogeneity of blood cell flux increased as indicated by the coefficient of variation (ratio of standard deviation to the mean flux), decreasing from 44.6% to 22.0%. These findings are in agreement with indirect data obtained by Knudsen et al42 for the human brain and closely correlate with those reported by Tyml43 for frog muscle. Hence, the major changes in capillary blood cell perfusion during high flow conditions are a shift from heterogeneous flow at rest to more homogeneous flow during stimulation accompanied by a drop in the number of low flow capillaries. Analogous to the original capillary recruitment concept, these low flow capillaries may be regarded as functional reserve capillaries. Following those lines, the classic concept of capillary recruitment merely appears as an extreme variant of this concept of functional reserve capillaries.

Since capillaries do not contain muscle cells, capillary diameter is usually assumed to be invariant. However, distensibility of capillaries has been reported to occur in bat wings,44 and capillaries in tenuissimus skeletal muscle of rabbits have been reported to dilate during reactive hyperemia after 2 minutes of complete aortic occlusion.45 Furthermore, in two recent studies, Atkinson et al46 and Duelli and Kuschinsky47 reported that cerebral capillary diameter as measured after perfusion fixation of brain tissue increases during hypercapnia. However, in these studies the influence of fixation artifacts cannot be completely ruled out. The present study, using a completely different methodological approach, also shows a slight but statistically significant increase in cerebral capillary diameter during hypercapnia. Thus, evidence is increasing that capillaries may no longer be regarded as rigid tubes. At present, it is not clear whether the observed changes in capillary diameter are due to passive dilation or whether some active regulatory mechanism is involved at the capillary level.

In conclusion, the results of the present study using CLSM in a closed cranial window preparation of the barbiturate-anesthetized rat in vivo indicate that cerebral capillaries are continuously perfused with plasma but may experience fluctuations in blood cell flow. When cerebral blood flow is augmented during hypercapnia, mean blood cell flux and velocity increase. Capillary perfusion with blood cells becomes more homogeneous, the number of poorly blood cell–perfused capillaries is decreased, and capillaries are dilated.

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