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Capillary Flow and Diameter Changes during Reperfusion after Global Cerebral Ischemia Studied by Intravital Video Microscopy

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Summary: The reaction of cerebral capillaries to ischemia is unclear. Based on Hossmann's observation of postischemic "delayed hypoperfusion," we hypothesized that capillary flow is decreased during reperfusion because of increased precapillary flow resistance. To test this hypothesis, we measured cerebral capillary erythrocyte velocity and diameter changes by intravital microscopy in gerbils. A cranial window was prepared over the frontoparietal cortex in 26 gerbils anesthetized with halothane. The animals underwent either a sham operation or fifteen minutes of bilateral carotid artery occlusion causing global cerebral ischemia. Capillary flow velocities were measured by frame-to-frame tracking of fluorescein isothiocyanate labeled erythrocytes in 1800 capillaries after 1-hour reperfusion. Capillary flow velocities were decreased compared to control (0.25 ± 0.27mm/s vs. 0.76 ± 0.45 mm/s; $P < 0.001$). Precapillary arteriole diameters in reperfused animals were reduced to 76.3 ± 6.9% compared to baseline ($P < 0.05$). Capillary diameters in reperfused animals (2.87 ± 0.97 μm) were reduced ($P < 0.001$) compared to control (4.08 ± 1.19 μm). Similar reductions of precapillary (24%) and capillary vessel diameters (30%) and absolute capillary flow heterogeneity indicate that delayed (capillary) hypoperfusion occurs as a consequence of increased precapillary arteriole tone during reperfusion. **Key Words:** Global cerebral ischemia—Cerebral capillaries—Flow velocity—Flow heterogeneity—Reperfusion.

INTRODUCTION

Secondary brain injury aggravates ischemic stroke (Hakamata et al., 1997; Gaehtgens, 1991; Pahlmark, 1993; Osuga and Hakim, 1994). Treatment or prevention of secondary or postischemic brain injury is a challenging task targeted to improve outcome after ischemic insult. The pathophysiology of secondary brain injury is multifactorial. Postischemic "delayed hypoperfusion" (Hossmann 1973; 1997) is one important factor that contributes to the development of secondary brain injury. Hossmann (1973; 1974) observed that global cerebral ischemia is followed first by a short phase of postischemic hyperemia that emerges into a secondary prolonged phase of delayed hypoperfusion. Others confirmed Hossmann's observation (Belayev et al., 2002; Uhl et al., 2000; Dijkhuizen et al., 1998; Spatz, 1996; Stummer, 1995; Pulsinelli et al., 1983). To date, the pathophysiology of delayed hypoperfusion remains poorly understood and requires further investigation to improve therapy and outcome after stroke (Hossmann, 1997). Only few mechanisms have been observed that could potentially cause delayed hypoperfusion. Belayev et al. (2002) observed postischemic stagnation of blood flow in postcapillary venules with thrombus / corpuscle formation and secondary hypoperfusion after middle cerebral artery occlusion. Uhl et al. (2000) found post-ischemic leukocyte activation. Delayed hypoperfusion may facilitate leukocyte-endothelium interaction by decreasing shear stress, or *vice versa,* leukocyte activation could increase blood viscosity and decrease CBF.

Vogel et al. (1999) described increased heterogeneity of the capillary Evans blue concentration in ischemic areas and hypothesized that this increased capillary flow heterogeneity during reperfusion was an index of microcirculatory disturbances. However, the role of capillaries in the pathogenesis of delayed hypoperfusion remains

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unknown. Baez et al. (1960) did not find significant diameter changes in mesenteric capillaries exposed to various pressures and concluded that capillaries behave as rigid tubules without the ability to adapt. More recent studies suggest a certain degree of capillary flexibility. Duelli and Kuschinsky (1993) reported capillary diameter changes in cerebral capillaries exposed to hypo- and hypercapnia. They concluded that brain capillaries exhibit a moderate degree of distensibility, with a decreased diameter during decreased perfusion.

Based on this finding of a variable capillary diameter, we hypothesized that capillary diameters are reduced during delayed hypoperfusion in close relation to decreased capillary flow. The objectives of this study were to assess capillary flow velocities, diameters, length and density during reperfusion following global cerebral ischemia *in vivo.* A gerbil cranial window model was used for the first time to study capillary flow velocities and capillary diameter changes by intravital video microscopy.

METHODS

Experimental groups

26 male mongolian gerbils (60–70g) underwent either 15 minutes of global cerebral ischemia (bilateral carotid artery occlusion) or a sham operation. All animals were divided randomly into four groups: group I_{sham} (n = 6), group $I_{ischemia}$ (n = 8), group II_{sham} (n = 6) and group $II_{ischemia}$ (n = 6). CBF, cerebral transit time (CTT) and precapillary arteriole diameters were assessed by *in vivo* microscopy and Laser Doppler flowmetry in animals of group I_{sham} and group $I_{ischemia}$ at baseline, 4, 8, 20, 40, 60, 90 and 120 minutes of reperfusion. Vascular resistance was estimated from the ratio mean arterial blood pressure MABP / CBF.

In separate animals (group II_{sham} / group $II_{ischemia}$), structural and functional parameters of cerebral subsurface capillaries were measured by intravital video microscopy. CBF of group $I_{ischemia}$ animals remained stable around 1-hour of reperfusion. We chose a five-minute window at 60 minutes of reperfusion for the analysis of capillary parameters during the phase of secondary hypoperfusion.

Animal preparation

The Institutional Animal Care and Use Committee approved the experimental protocol used in these studies. Anesthesia was induced with halothane by face mask (4% in 30% O2, 70% N2, 1L/min), maintained with 2% during surgical procedures and reduced to 0.5% throughout the experimental measurements. Rectal temperature was maintained at 37°C with a feedback-controlled heating blanket. The tail artery was cannulated for continuous monitoring of the mean arterial blood pressure (MABP) and arterial blood gases (ABG). The left femoral vein was cannulated for the infusion of fluorescent markers. After a midline incision in the neck, both carotid arteries were exposed and encircled gently with a 7–0 monofil suture without compromising blood flow. The head was secured in a stereotactic frame and a right parietal craniotomy (3 × 4mm) was performed for intravital microscopy. The dura remained intact as previously described by Uhl et al. (2000). Any laceration of the dura or any bleeding excluded the animal from the study. To keep the dura moist and transparent, the dura was superfused with 37°C warm physiological saline. Brain temperature was measured with an ear bar thermocouple probe and maintained with a near-infrared radiator (Brambrink et al., 1999).

In groups I_{sham}/$I_{ischemia}$, an addititional, smaller parietal craniotomy was performed on the left side for continuous local cerebral blood flow (lCBF) measurements with the Laser Doppler (LaserFlo, Vasamedics, St. Paul, MN, USA). After surgical preparation, the animals were positioned on a computer controlled microscope stage for repeated analysis of identical segments of cerebral blood vessels. Baseline conditions were measured during a control phase of 25 minutes. To achieve 15 minutes of global cerebral ischemia, the 7–0 suture was tightened with a fifteen-gram weight. Ischemia was confirmed by Laser Doppler flowmetry and/or significant rise in systemic arterial blood pressure. Sham animals were treated similarly, but the suture was not tightened. Fifteen minutes later, the suture was removed from both sham and ischemia animals and reperfusion was initiated in the experimental animals.

Transit time and Laser Doppler flowmetry (group I_{sham}/group $I_{ischemia}$)

We used the cerebral transit time (CTT) measured by *in vivo* microscopy to analyze CBF changes in group I_{sham} and group $I_{ischemia.}$ CTT was measured according to a technique described by Liebetrau et al. (2002). A Zeiss microscope (Axiotech, Oberkochen, BW, Germany) with a water immersion objective (x20) was used for *in vivo* microscopy. To reduce light intensity and artefacts, images were recorded with a Silicon Intensifier Target (SIT) camera (C2400, Hamamatsu Photonics, Herrsching, Germany). A halogen lamp (6V) with beamsplitter filter blocks and gray filter was used for epi-illumination of pial vessels. A pial arteriole (30 – 60 μm) next to a pial vein (30 – 60 μm) was captured in the same field of view. Rhodamine 6 G (Sigma, St. Louis, MO, USA) was injected intravenously over one second as a bolus (0.01%; 0.02ml/ 100 g b.w.). The SIT camera recorded with a frequency of 25 frames per second. This allowed off-line intensity-change analysis in pial vessels every 40 milliseconds with the digital image analysis program capimage (Dr. Zeintl, Heidelberg, Germany). The CTT was calculated as the latency of signal intensity increase between the pial arteriole and its neighboring vein (Liebetrau et al. 2002). With every CTT measurement, we also analyzed precapillary arteriole diameters during contrast enhancement off-line with capimage. CBF was monitored by stationary Laser Doppler flowmetry over the left parietal cortex. Mean arterial blood pressure, rectal temperature and tympanic membrane temperature were continually monitored. Arterial blood gasses were measured at baseline and two hours of reperfusion.

Videomicroscopy of cerebral capillaries (groups II_{sham}/group $II_{ischemia}$)

A total number of 1800 capillaries were studied in animals of groups II_{sham}/$II_{ischemia}$ at 1-hour of reperfusion. Sufficient spatial resolution could be achieved with a CCD camera, FITC beam-splitter filter blocks and epi-illumination with an adjustable 100 W Xenon lamp (Zeiss, Oberkochen, BW, Germany) (Fig. 1). To avoid artefacts secondary to repeated exposure to fluorescent light, capillary measurements were performed only at one point of time. Light intensity, amount of the injected fluorescence marker and time of exposure were strictly minimized to avoid illumination artefacts. Red blood cells (RBCs) were labeled *in vitro* prior to the experiment according to Hudetz et al. (1995). Briefly, a gerbil was sacrificed, full blood was harvested and centrifugated for 10 minutes. Buffy coat and plasma were removed and the RBCs were washed in

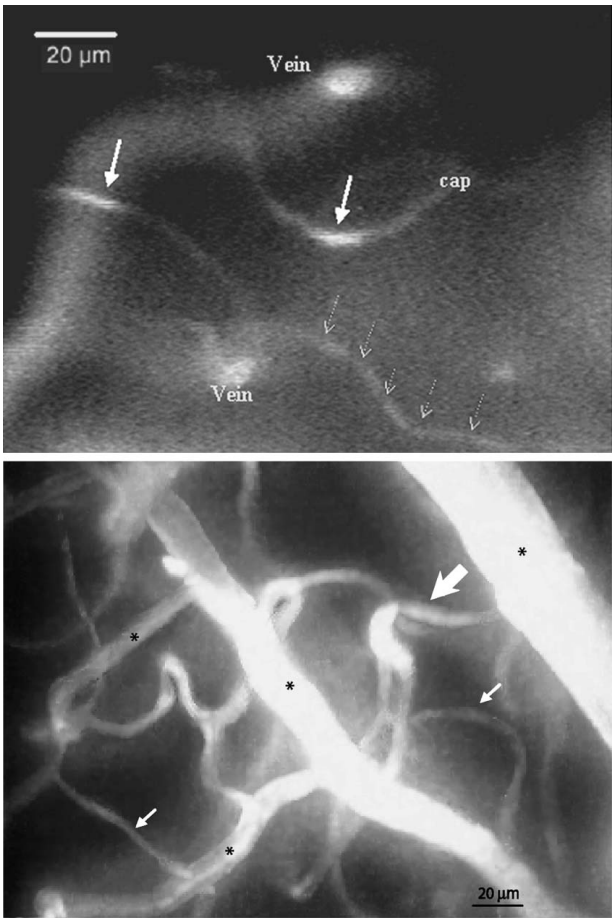

FIG. 1. Top. Typical video-frame capturing capillaries and post-capillary venules. White solid arrows mark moving FITC labeled red blood cells (FRBCs) in a low flow area (0.4 mm/s). FRBCs appear elongated as they moved while the image was taken (40 ms). The apparent length of the FRBCs reflects their flow velocity. Interrupted white arrows mark non-labeled erythrocytes appearing as short black gaps within FITC labeled plasma. Bottom. Three-dimensional reconstruction of the capillary network from fifty video images. Exchanging capillaries are the smallest units of the capillary network (small arrows). Throughfare channels are high flow capillaries with a larger diameter (large arrow). A branch of the collecting veins (asterisk) has a similar size compared to the throughfare channel. The vessel's three-dimensional course allows the differentiation between capillary and venule.

glucose-saline buffer (pH 7.4). RBCs were then incubated for two hours with 9 mg fluorescein isothiocyanate (FITC) per ml blood and washed in buffer at least five times. The harvested fluorescent RBCs (FRBCs) were diluted with normal saline to a hematocrit of 45%. Three drops of citrate-phosphate-dextrose buffer were added for conservation of the sample. Immediately before injection, the sample was washed again in buffer two times and diluted to a hematocrit of 45% with normal saline. At forty-five minutes of reperfusion (fifteen minutes prior to the microscopy measurements of capillaries), FRBCs were injected intravenously (0.15 mL suspension / 100g). Additionally, a 0.2% FITC dextran solution (0.15ml/100g b.w.) was injected for better visualization of the capillary network. An arterial blood gas analysis was performed immediately after infusion of FRBCs. Rectal temperature, tympanic membrane temperature, and arterial blood pressure were continuously monitored.

Identification of capillaries

Capillaries were identified by the presence of single-file erythrocyte flow and by their tortuous and branching pathway (Hudetz et al., 2000). The characteristic three-dimensional gestalt of the capillary network becomes apparent in the motion picture. Various optical planes at different depths in the brain appear in the same field of view during spontaneous pulsation of the brain and during focus adjustments. For illustration, we simulated the motion picture effect by averaging fifty consecutive video images of the same field of view with Corel Photo Paint. Using the functions window, docker, object and merge mode 'if lighter' the imaging software selects the brightest pixel of all images at identical positions to create a new 'merged' image pixel by pixel. Only bright structures such as vessels in the focus plane were transferred into the new fused image (Fig. 1). Capillaries were identified by their typical three-dimensional course rather than by a certain threshold of diameter size or flow velocity.

Data analysis

The videotapes were analyzed blinded and off-line frame-by-frame at a total magnification of x 1400. A total of 5400 FRBC velocities were measured. Six capillaries were assessed per field of view, 150 capillaries per animal. FRBC velocities were measured 3 times in 3 to 5 second intervals. In order to measure the capillary diameter, the first step was to select the frame capturing a capillary's segment with the minimal "blooming effect", i.e. maximal intensity change over the shortest possible distance across the capillary borders (Fig. 1). Often no such frame could be identified and therefore, we only measured 120 capillaries per animal. One capillary border was marked with the imaging software capimage along the "line of maximal intensity change". The imaging software measures then the distance of a vertical connection line (the capillary diameter) to the opposite "line of maximal intensity change", the opposite capillary border. Five measurements for each capillary were averaged. A total of 7200 diameters were measured. The capillary length was defined as the distance from one branch point to the next. None or only one branch point was visible in capillaries leaving the optical plane. Consequently, for length measurements we used only capillaries nearly parallel to the optic plane. The capillary density was measured as the sum of the lengths of all visible capillary segments divided by the field of view (cm/cm^2). (Uhl et al., 2000)

Statistics and calculations

Data are presented in the text as mean and standard deviation. Standard error is used in plots. Differences were regarded as statistically significant at $P < 0.05$. Data were analyzed with the Student's t-test. Flow velocities at 60 minutes of reperfusion, diameter and heterogeneity were analyzed with the Mann-Whitney Rank Sum test. The coefficient of variation for red blood cell velocities (RBCV) was calculated as SD/mean (Kuschinsky, 1996). The ratio of the relative MABP [% baseline] divided by lCBF [%baseline] was used to estimate changes of the relative cerebral vascular resistance (CVR) [% baseline]. Cerebral vascular resistance (CVR) could not be calculated directly because intracranial pressure (ICP) was not measured. However, previous experiments (Morimoto et al., 1996; Miller et al., 1980) demonstrate that ICP remains at baseline during reperfusion after global cerebral ischemia. ICP was increased by 5 mmHg only during hyperemia (Morimoto et al. 1996). Thus, in our study we assumed that relative MABP [% baseline] nearly equals relative cerebral perfusion pressure (CPP) [% baseline] and the ratio "MABP/lCBF" estimates

CVR. During hyperemia, CVR might be overestimated by approximately 6.7% (5 mmHg/75 mmHg).

RESULTS

Systemic Parameters (compared to control)

Mean arterial blood pressure (MABP) remained constant in control animals (73.9 ± 3.0 mmHg, n = 12) but increased in experimental animals after bilateral carotid artery occlusion (124.1 ± 11.2 mmHg; n = 14; $P < 0.001$). At one hour of reperfusion, MABP was slightly higher in postischemic animals than in sham-operated animals. Hematocrit and arterial blood gasses remained stable (Table 1). The tympanic membrane temperature (TMT) in sham animals was constant during the experiment (36.7 ± 0.3°C). In ischemic animals, TMT dropped from a baseline of 36.4 ± 0.7°C to 35.6 ± 0.8°C at the end of the ischemic period ($P < 0.01$), reached a peak at hyperperfusion and dropped to 35.9 ± 0.4°C at one hour after reperfusion ($P < 0.01$).

CBF by Laser Doppler and cerebral transit time (CTT) (groups I_{sham}/$I_{ischemia}$)

Throughout the experiment, there were no significant changes of lCBF in group I_{sham} animals (n = 6, Fig. 2). LCBF was reduced to 4.9 ± 2.4% ($P < 0.001$) during global cerebral ischemia in animals of group $I_{ischemia}$ (n = 8). After 8 to 10 minutes of reperfusion, there was a short phase of hyperperfusion to 128.7 ± 10.0% of baseline, which was followed by a long lasting hypoperfusion until the end of the experiment. After 1-hour of reperfusion, the hypoperfusion reached a stable plateau with lCBF of 63.1 ± 11.6% of baseline values ($P < 0.01$). The CTT ranged from 300 milliseconds to 1000 milliseconds at baseline. The CTT remained constant in I_{sham} animals at all times (Fig. 2). During early reperfusion in experimental animals, the CTT was elevated to 309 ± 39% of baseline ($P < 0.001$), dropped to 85 ± 18% of baseline during hyperperfusion and increased progressively to 250 ± 37% at 1-hour of reperfusion ($P < 0.001$) and 296 ± 44% at 2-hours of reperfusion ($P < 0.001$).

Estimated vascular resistance and precapillary arterioles (groups I_{sham}/$I_{ischemia}$)

The precapillary arteriole diameters ranged from 30 to 60 μm at baseline and remained unchanged during the experiment in sham operated animals. In experimental animals, the arterioles were dilated during early reperfusion prior to hyperemia (Fig. 2). The ratio MABP / lCBF (estimated vascular resistance) was increased at the corresponding time ($P < 0.05$). The arteriolar diameters then returned to baseline during hyperemia when the ratio MABP / lCBF dropped below baseline. Thereafter arteriolar diameters were reduced to 76.3 ± 6.9% of baseline ($P < 0.001$) at 1-hour of reperfusion with an increased ratio MABP / lCBF ($P < 0.05$).

Capillaries after 1-hour of reperfusion (group II_{sham}/group $II_{ischemia}$)

The mean capillary FRBC velocity in the sham group was 0.76 ± 0.45 mm/s (Fig. 3, Table 2), ranging from no flow in six capillaries (0.7%) to the maximum velocity of 3.0 mm/s. The mean coefficient of variance (var_{coeff} = SD/mean) calculated according to Kuschinsky (1996) was 50 ± 13%. The mean capillary diameter was 4.08 ± 1.19 μm (Fig. 3), capillary length 59.8 ± 21.0 μm and capillary density 121.7 ± 21.0 cm/cm^2.

In experimental animals, the average capillary flow velocity at 1-hour of reperfusion after fifteen minutes of global cerebral ischemia was significantly decreased to 0.25 ± 0.27 mm/s compared to control ($P < 0.001$, Fig. 3). No flow was found in 58 capillaries (6.4%) that contained non-moving usually non-labeled red blood cells. The capillary diameter was significantly reduced to 2.87 ± 0.97μm compared to control ($P < 0.001$, Fig. 3). Capillary flow heterogeneity was significantly increased to 101 ± 24% in the ischemia group ($P < 0.01$). Capillary length (59.2 ± 19.8 μm) and density (120.2 ± 339 cm/cm^2) did not change significantly (Table 2).

DISCUSSION

Model

The major advantage of the gerbil cranial window compared to the rat closed cranial window model (Morii et al., 1986) is the free view of the subsurface capillaries in the cerebral cortex without the need to remove the dura. Superfusion of the brain with artificial CSF with equilibrated pH is unnecessary because the superfusate (physiological saline) is separated from the brain surface

TABLE 1. *Systemic parameters at baseline conditions, 1-hour and 2-hours of reperfusion in Mongolian gerbils (Mean ± SD; n = 24)*

	Sham			Ischemia		
	Baseline	1h Rep	2h Rep	Baseline	1h Rep	2h Rep
MAPB [mmHg]	73.3 ± 2.52	74.8 ± 1.72	73.3 ± 1.25	72.7 ± 4.84	76.0 ± 1.41	70.5 ± 11.86
$Temp_{tymp}$ [°C]	36.1 ± 0.7	36.2 ± 0.5	36.2 ± 0.5	36.6 ± 0.2	36.0 ± 0.1	35.9 ± 0.2
Arterial pH	7.32 ± 0.02	7.34 ± 0.04	7.32 ± 0.02	7.36 ± 0.01	7.35 ± 0.05	7.31 ± 0.01
PCO^2 [torr]	44.5 ± 1.0	42.82 ± 4.00	41.7 ± 0.7	43.1 ± 1.5	44.53 ± 1.84	42.9 ± 2.2
PO_2 [torr]	109.2 ± 5.1	126.8 ± 36.1	124.8 ± 4.9	119.4 ± 6.6	114.0 ± 24.4	144.3 ± 19.3
HCO^3 [mmol/l]	21.8 ± 0.9	22.4 ± 2.0	20.5 ± 0.9	23.7 ± 1.0	23.5 ± 1.4	21.0 ± 1.1
Hct [% RBC]	43.2 ± 2.8	45.2 ± 2.0	40.6 ± 1.6	44.7 ± 3.2	45.5 ± 3.2	45.4 ± 3.9

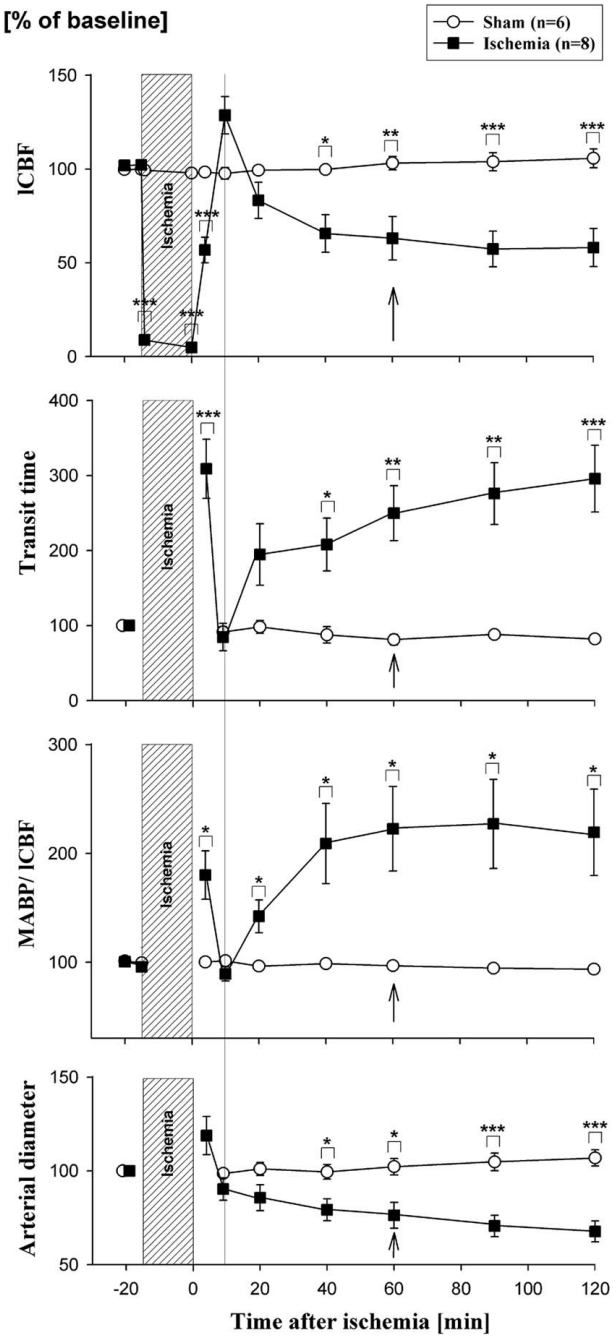

FIG. 2. Local cerebral blood flow (lCBF), cerebral transit time (CTT), ratio of mean arterial blood pressure (MABP) and lCBF and precapillary arteriolar diameters displayed as percentage of baseline against time in minutes. Values represent mean ± SEM; n=6. Data were analyzed by Student's t-test (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$). LCBF was reduced to 4.9 ± 0.9% during ischemia. After a brief hyperemic phase at 8 to 10 minutes of reperfusion, a long lasting hypoperfusion occurred. CTT is inversely proportional to lCBF. The ratio MABP / lCBF was calculated as 100 x MABP [% baseline] divided by lCBF [% baseline] as estimate of cerebral vascular resistance (Morimoto et al., 1996). Precapillary diameters decreased during hypoperfusion. We chose a 5-minute window at 60 minutes of reperfusion to investigate capillary changes (Fig. 3). At this time (arrow in Fig. 2), CBF and precapillary arteriolar diameters were reduced; CTT and vascular resistance are increased. The results suggest that increased precapillary arteriolar tone contributes to increased flow resistance causing delayed hypoperfusion.

by the dura. Using this model, we were able to demonstrate for the first time capillary diameter changes or capillary flow velocities by tracking fluorescence labeled erythrocytes with high spatial resolution. Three-dimensional reconstruction allows network analysis and better identification of capillaries.

Precapillary arteriolar tone during reperfusion

The increase in estimated cerebral vascular resistance combined with precapillary arteriolar diameter reduction at one hour of reperfusion is evidence for Hossmann's assumption about the pathomechanism of delayed hypoperfusion (Hossmann, 1997): Increased precapillary

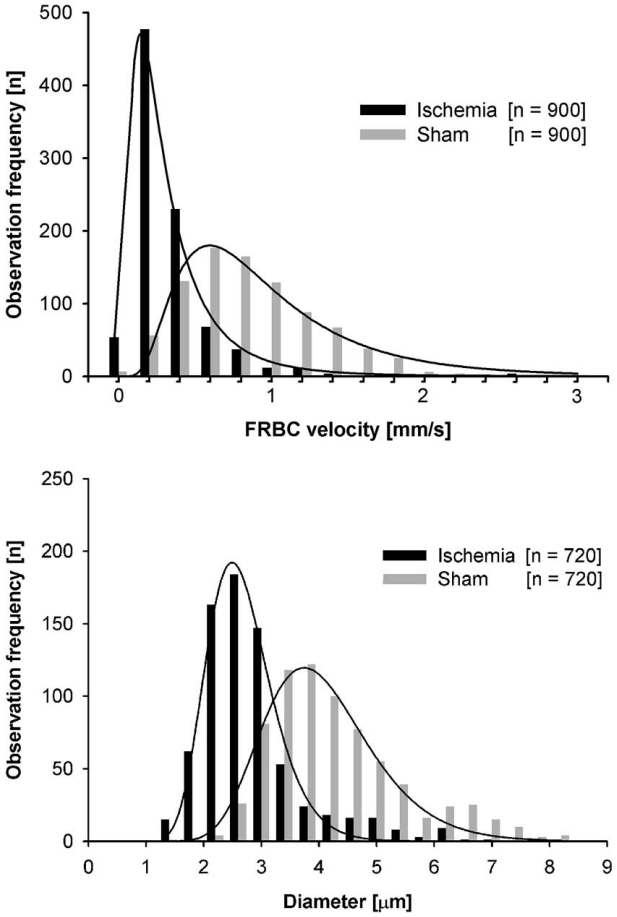

FIG. 3. Capillary measurements at 1 hour of reperfusion. Top. FITC labeled Red Blood Cell (FRBC) flow velocities [mm/s] are shown in classes on the x axis: 0 mm/s; 0 mm/s < x ≤ 0.2 mm/s; 0.2 mm/s < x ≤0.4 mm/s;...; 2.8 mm/s < x ≤3.0 mm/s. The absolute observation frequencies are displayed on the y axis. Fifty-eight capillaries (6.7%) showed no flow during delayed hypoperfusion. The mean flow velocity was reduced from 0.76 ± 0.45 mm/s in sham animals to 0.25 ± 0.27 mm/s in postischemic animals ($P < 0.001$, Mann-Whitney Rank Sum Test). The coefficient of variance (SD □ × 100/mean) as a measure of flow heterogeneity was increased in postischemic animals to 101 ± 24% compared to 50 ± 13% in sham animals ($P < 0.01$; T-test). Bottom. Capillary diameters are displayed on the x axis in μm and the absolute observation frequencies on the y axis. We observed postischemic capillary diameter reduction at one-hour reperfusion ($P < 0.001$, Mann-Whitney Rank Sum Test).

TABLE 2. *Summary of data obtained from subsurface capillaries in vivo at one hour of reperfusion following 15 minutes global cerebral ischemia. No structural change of the capillary network (density, length), but significant reduction of FITC labeled Red Blood Cell (FRBC) flow velocities and capillary diameters were observed.*

Capillary parameters	Velocity [mm/s]		Diameter [μm]		Length [μm]		Density [cm/cm^2]	
Exp. group	Sham	Ischemia	Sham	Ischemia	Sham	Ischemia	Sham	Ischemia
Mean	0.76	0.25	4.08	2.87	59.8	59.2	121.7	120.2
Stand Error	0.02	0.01	0.04	0.04	1.57	1.47	4.18	4.38
Stand Dev	0.45	0.27	1.19	0.97	21.0	19.8	32.4	33.9
Minimum	0	0	1.36	1.20	20.2	15.5	44.0	45.6
Maximum	3.00	2.58	9.32	7.12	145.5	121.4	205.5	190.0
N	900	900	720	720	180	180	60	60

vessel tone results in secondary hypoperfusion. An unphysiological imbalance of vasoactive substances such as thromboxane A2, prostaglandins, endothelin, prostacyclin and nitric oxide may account for this phenomenon (Lockinger et al., 2001; Cavaglia et al, 2001; Pinard et al., 2000; Laufs et al., 2000; Bolanos et al., 1999; Yasuma et al, 1997; Spatz et al, 1996; Huang et al., 1996; Ngai and Winn, 1995; Huang et al., 1994; Kito et al., 1993). To date however, no study has observed continuous development of precapillary arteriole diameter reduction during reperfusion in the brain *in vivo.* Postischemic hypoperfusion and increased vascular tone in precapillary arterioles during reperfusion was observed in a rabbit lung ischemia-reperfusion model (Lockinger et al., 2001). Hart et al. (1978) found decreased vessel diameters and vessel wall thickening in no-reflow areas. Wisniewski et al. (1995) noted a vasoconstrictive response with thickening of the vessel basement membranes, increased endothelial microfilaments and vesicular profiles during reperfusion at 6, 12 and 24 hours after global cerebral ischemia. Their studies support our results suggesting increased precapillary tone during delayed hypoperfusion.

Postischemic capillary diameter reduction

Our studies demonstrated for the first time that capillary diameters are reduced during delayed hypoperfusion. Only few other investigators studied capillary diameter changes during ischemia/reperfusion. Mchedlishvili et al. (1997) assessed capillary diameter reduction during CBF reduction to 57% of baseline after carotid artery occlusion. They report a reduction of the capillary diameter of 9.7% in capillaries perfused with red blood cells and 37.5% in capillaries perfused only with plasma. Studies using electron microscopy revealed postischemic swelling of endothelial microvilli and perivascular glia that could account for the decreased capillary diameter during reperfusion (Fischer et al., 1977; Merrill, 1969; Chiang et al., 1968; Dietrich et al., 1984). Fischer et al. (1977) noticed a significant decrease in capillary diameters after 30 minutes of reperfusion with an increased number of small capillaries in no reflow areas.

Despite observed capillary wall changes, Fischer et al. (1977) suggested that the cause of decreased perfusion during reperfusion is rather functional than structural. The question still remains whether the observed postischemic capillary diameter reduction is active or passive. Some authors have suggested *venous* plugging by corpuscle formation, thrombosis and leukocyte activation as a mechanism of postischemic hypoperfusion (Belayev et al., 2002; Ritter et al., 2000; Uhl et al., 2000; Bottiger et al., 1997; Obrenovitch and Hallenbeck, 1985). However, capillary distension would be the expected consequence of this phenomenon. The constriction of precapillary arterioles to approximately the same degree as postischemic capillaries (70% compared to control/baseline) suggests passive decrease in capillary perfusion/diameter as a consequence of increased precapillary arteriole tone. Cerebral precapillary arterioles may act as "capillary network controllers" in analogy to Sweeney's observations in the hamster cremaster muscle (Sweeney et al., 1990).

An active component of the capillary endothelium however, cannot be excluded. Few reports mention contractile pericytes at the capillary level (Tilton et al, 1979, Hammersen et al, 1984; Chen et al., 1999). The endothelium itself has an actin/myosin based contractile system (Gotlieb et al, 1988; Kempski et al., 1987). Chen et al. (1999), for example, demonstrated compaction of F-actin filaments in human cerebral endothelial cells modulated by intracellular calcium changes. Additionally, Boswell et al. (1992) found a smooth muscle-like contractile mechanism for endothelial cell contraction *in vitro.* These studies support the hypothesis that the imbalance of metabolic mediators during reperfusion may act on capillary endothelium directly and contribute to capillary diameter reduction during reperfusion.

Postischemic capillary diameter reduction observed in our studies affects capillary flow resistance directly (Hagen-Poiseuille) and may also impair capillary rheology. A thin parietal plasma layer surrounds erythrocytes (Mchedlishvili et al., 1995) that act as a sliding film between erythrocyte and endothelium. Blood viscosity decreases with decreasing vessel diameter and reaches a

minimum (optimum) at the capillary level (Fahraeus–Lindqvist effect). However, the effect is reversed in vessels with a diameter of less than 4μm when viscosity increases (Hudetz et al., 1989). This reversal of the Fahraeus Lindqvist effect may be explained by a breakdown of the parietal plasma in capillaries below 4μm. Kuke et al. (2001) report increased blood viscosity to 140% of baseline after 1-hour of reperfusion during cerebral ischemia. Thus, postischemic capillary diameter reduction found in our studies may contribute to an increase of capillary blood viscosity during reperfusion.

Capillary flow heterogeneity

Our studies revealed an increase in relative capillary flow heterogeneity after ischemic reperfusion. Flow heterogeneity is an important feature of cerebral perfusion that may reflect the constantly changing metabolic demand of brain tissue with regulatory response of the capillary network (Villringer et al., 1994; Dirnagl et al., 1991; Villringer et al., 1991; Hudetz et al., 1993; Hudetz et al., 1989; Hertz and Paulson, 1980; Abounader et al., 1995). However, little is known about flow heterogeneity in a low flow state such as delayed hypoperfusion, and speculations are controversial. On the one hand, studies document a constant flow homogenization with increasing CBF during hypercapnia (Aboundader et al., 1995; Villringer et al., 1994). Extrapolation of these data suggests increase of flow heterogeneity during hypoperfusion. Indeed, Vogel et al. (1999) found increased flow heterogeneity during reperfusion after middle cerebral artery occlusion in areas of decreased blood flow, indicating compromised microcirculation. On the other hand, Hudetz et al. (1994) predicted flow homogenization in a hypoperfused network. Vessels with low flow are converted into "plasma channels" as a result of non-linear partitioning at their entrance (Hudetz et al., 1989). Flow heterogeneity during physiological cerebral perfusion may also indicate the presence of a "functional reserve flow" in the absence of capillary recruitment (Hudetz et al., 1994). The flow reserve could be utilized by the partial redistribution of flow from throughfare channels to poorly perfused capillaries during hypoperfusion (Luebbers et al., 1978). The redistribution would result in capillary flow homogenization during hypoperfusion (Hudetz et al., 1994).

Duling et al. (1987) differentiate between an "absolute" measure for sample heterogeneity (SD) and "relative" measure such as the variation coefficient (SD/mean). Our results show a decrease of absolute flow heterogeneity (reduced SD) with an increase of relative flow heterogeneity (increased ratio SD/mean) during reperfusion. The divergence of absolute and relative flow heterogeneity is due to significant flow reduction (greater reduction of the mean flow velocity than of its SD). This indicates alteration of the physiological capillary flow pattern with similar low flow in most capillaries.

CONCLUSION

Delayed hypoperfusion after global cerebral ischemia is a pathophysiological process with progressively increasing precapillary arteriolar tone and consequent breakdown of the capillary microcirculation. Decreased capillary flow may be aggravated by postischemic capillary diameter reduction, reversal of the Fahraeus-Lindqvist effect and increased capillary blood viscosity. The decreased number of high flow capillaries with divergent absolute and relative flow heterogeneity indicates alteration of the physiological capillary flow pattern with loss of capillary flow reserve. Thus, reversal of delayed hypoperfusion should be part of a therapeutic strategy to alleviate secondary brain injury after stroke.

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