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Spatial flow-volume dissociation of the cerebral microcirculatory response to mild hypercapnia

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The spatial and temporal response of the cerebral microcirculation to mild hypercapnia was investigated via two-photon laser-scanning microscopy. Cortical vessels, traversing the top 200 μm of somatosensory cortex, were visualized in α-chloralose-anesthetized Sprague–Dawley rats equipped with a cranial window. Intraluminal vessel diameters, transit times of fluorescent dextrans and red blood cells (RBC) velocities in individual capillaries were measured under normocapnic ($PaCO_2$ = 32.6 ± 2.6 mm Hg) and slightly hypercapnic ($PaCO_2$ = 45 ± 7 mm Hg) conditions. This gentle increase in $PaCO_2$ was sufficient to produce robust and significant increases in both arterial and venous vessel diameters, concomitant to decreases in transit times of a bolus of dye from artery to venule (14%, $P < 0.05$) and from artery to vein (27%, $P < 0.05$). On the whole, capillaries exhibited a significant increase in diameter (16 ± 33%, $P < 0.001$, $n = 393$) and a substantial increase in RBC velocities (75 ± 114%, $P < 0.001$, $n = 46$) with hypercapnia. However, the response of the cerebral microvasculature to modest increases in $PaCO_2$ was spatially heterogeneous. The maximal relative dilatation (range: 5–77%; mean ± SD: 25 ± 34%, $P < 0.001$, $n = 271$) occurred in the smallest capillaries (1.6 μm–4.0 μm resting diameter), while medium and larger capillaries (4.4 μm–6.8 μm resting diameter) showed no significant changes in diameter ($P > 0.08$, $n = 122$). In contrast, on average, RBC velocities increased less in the smaller capillaries (39 ± 5%, $P < 0.002$, $n = 22$) than in the medium and larger capillaries (107 ± 142%, $P < 0.003$, $n = 24$). Thus, the changes in capillary RBC velocities were spatially distinct from the observed volumetric changes and occurred to homogenize cerebral blood flow along capillaries of all diameters.
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Keywords: Cerebral blood flow; Brain; Red blood cell velocity; Hypercapnia; Two-photon microscopy

Introduction

The most prominent methodologies for non-invasive imaging of human brain function – functional magnetic resonance, positron emission tomography, and near-infrared spectroscopy – all rely on tight coupling between focal cerebral hemodynamics and neuronal activity (Raichle, 2003). A detailed understanding of the mechanisms of local cerebral circulatory regulation is thus critical for the establishment of accuracy and range of the applicability of these techniques.

Like the neuronal network itself, the cerebral vascular tree exhibits both hierarchy and spatial specialization. At rest, local blood flow varies as much as 18-fold between the different regions of a rat brain (Fenstermacher et al., 1991), likely resulting from regional differences in capillary density (Patlak et al., 1984) as well as from transit time variations (Rosen et al., 1991). Moreover, CBF undergoes local (in addition to global) regulation so that, on a sub-millimeter scale, maps of hemodynamic changes closely follow those of neuronal activity (at least at the columnar level) under a variety of conditions (Cox et al., 1993; Woolsey et al., 1996; Malonek and Grinvald, 1996; Duong et al., 2001; Logothetis, 2002). A growing number of studies have suggested that both the density of the capillary beds as well as the amplitude and the temporal evolution of blood flow response to functional activation follow the cortical neuronal architecture (Cox et al., 1993; Gerrits et al., 2000; Harrison et al., 2002; Silva and Koretsky, 2002; Lu et al., 2004). While the existence of capillary level structures for very fine hemodynamic regulation has been demonstrated in various species (Ehler et al., 1995; Rodriguez-Baeza et al., 1998; Harrison et al., 2002), the spatial limit of hemodynamic adjustments remains unclear and is a subject of current interest (Lauritzen, 2001), as it dictates the theoretical limit on the functional specificity of flow-weighted neuroimaging techniques.

Indeed, it is the microcirculatory CBF control that is of most interest to brain function investigations due to the proximity of the capillary network to the activated parenchyma, and thus it is crucial to understand how capillary diameter and red blood cell (RBC) velocities are regulated. While a heterogeneous profile of microcirculatory CBF adjustments has long been suspected (Rosenblum, 1965), data on the spatial pattern of microvascular flow regulation in the brain have been scarce, likely due to the intrinsic difficulty of achieving the required spatial resolution in vivo. Conventionally, the pial microvessels have been directly

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observed via intravital microscopy in animals equipped with closed cranial windows (Navari et al., 1978) and the vessel diameters measured by a calibrated video microscaler (Morii et al., 1986; Wagerle and Degiulio, 1994; Parfenova et al., 1995; Ishimura et al., 1996; Hudetz, 1997; Takenaka et al., 2000, 2003; Iliff et al., 2003; Xu et al., 2004). Although important information on the effects of a range of vasoactive agents has been collected, these studies have been constrained to the pial surface and, typically, to vessels with diameters larger than 10 μm.

The advent of scanning confocal microscopy enabled imaging of vessels below the cortical surface, nonetheless with limited penetration capabilities. Microvascular CBF increases elicited by hypercapnia (Villringer et al., 1994; Seylaz et al., 1999), as well as decreases under ischemic conditions (Morris et al., 1999; Pinard et al., 2000), have been investigated with scanning confocal microscopy. Two-photon laser-scanning microscopy, on the other hand, brought in an additional number of important advantages, particularly for in vivo biological applications, that have afforded widespread use (Denk et al., 1990) and made significant impact on in vivo microscopy. Recently, the applicability of two-photon microscopy to the investigation of cerebral microcirculation has been demonstrated in rat somatosensory cortex following vibrissae or hindlimb stimulation (Kleinfeld et al., 1998), rat dorsal olfactory bulb during odor stimulation (Chaigneau et al., 2003), mouse somatosensory cortex during bicuculline-induced focal epileptiform activity (Hirase et al., 2004) as well as freely moving awake rats (Helmchen et al., 2001).

The purpose of the present work was to investigate the spatial and temporal evolution of microvascular CBF regulation using two-photon laser-scanning microscopy. To elicit changes in vessel diameter and in RBC velocities, we employed mild hypercapnia while imaging the microcirculation of the rat somatosensory cortex. Mild hypercapnia was chosen as an effective and simple way to elicit robust increases in global resting blood flow, thereby obviating the need for localization of the affected region and hence greatly facilitating the use of two-photon microscopy. Using fluorescently labeled dextrans, we quantified the changes in transit times from arteries to venules and veins. The concomitant vasodilatation was quantified by estimating the diameter of arteries, veins and capillaries from stack maximum intensity projections. Special attention was given to capillaries. The present experiments yield new insight into the spatial distribution of CO_2-induced changes in hemodynamics, in terms of both RBC velocity and intraluminal diameter, and provide detailed information on microvascular CBF regulation.

Materials and methods

Animal preparation

Twenty male Sprague–Dawley rats, weighing 146–300 g, were anesthetized with isoflurane (3% for initial induction and 1.5% for maintenance) in O_2-enriched medical air, orally intubated and mechanically ventilated throughout the remainder of the experiments (with 1.5% isoflurane concentration). Respiratory parameters, including end-tidal CO_2 levels, were carefully monitored by a capnograph (BCI 300 Capnocheck, BCI, Inc, Waukesha, Wisconsin). Rectal temperature was monitored and maintained at 37.0°C by means of a feedback-controlled warm water bath. The right femoral artery and vein were cannulated for blood gas analyses and intravenous administration drugs. After surgery, all wounds were treated with 2% lidocaine and closed.

Closed cranial window

The rats were secured to a custom-built stereotaxic equipped with plastic ear pieces and a bite-bar. The dorsal area of the scalp was shaved, and a rostral-to-caudal incision was made to expose the skull. Connective tissue above the skull was removed, and local bleeding was controlled either by cauterization or by coating the skull with bone wax. Some portions of the temporal muscle on the side of the cranial window were excised, and the location of a cranial window over the primary somatosensory cortex was determined based on stereotaxic coordinates (center at 4 mm lateral to bregma, 0 mm caudal to bregma, diameter 5 mm). Using a dremel with a burr bit, the perimeter around the window was carefully thinned until pial vessels were visible. Ice-cold saline solution was constantly applied during drilling to avoid overheating of the skull. The area of skull within the perimeter was then removed with no. 7 forceps. In most cases (animal body weight BW <250 g, $n = 14$), the dura matter below the cranial window remained intact and was left in place. In a few cases, however (BW ≥250 g, $n = 6$), it was necessary to remove the dura matter by careful dissection, leaving the pial surface of the cortex exposed. Once the craniotomy was completed, the ear bars were removed and a metal frame was fixed around the craniotomy site with dental cement and held in place by custom made metal bars attached with pins on either side of the frame (Yoder and Kleinfeld, 2002). The bars were secured to the stereotaxic stage by the ear bar holders and adjusted until the cranial window was oriented horizontally. The well over the cranial window was then filled with 1% wt./vol. agarose and covered with a custom cut Corning glass #1 cover slip.

The animal and sample stage were then moved and fixed to the microscope stage. Anesthesia was switched from isoflurane to an intravenous solution of α-chloralose (80 mg/kg BW initial bolus, followed by a constant infusion of 27 mg/kg BW/h) supplemented with pancuronium bromide as needed (2 mg/kg BW/90 min) to minimize motion artifacts. To visualize vessels, blood plasma was fluorescently labeled by an intravenous injection of 150–300 μl of 0.5 mg/ml rhodamine-labeled dextran (70,000 MW) in phosphate-buffered saline.

Blood gases and hypercapnia

Blood gases were carefully monitored and adjusted throughout experiments to ensure accurate normocapnic and hypercapnic conditions. Ventilation parameters (rate, tidal-volume and oxygen concentration in gas mixture) were adjusted to correct PaO_2 and $PaCO_2$. Whenever necessary, sodium bicarbonate was injected intravenously to correct for acidic blood pH. Once the blood gases were in physiological range (normocapnic condition), the imaging commenced. Blood gases were periodically sampled to ensure physiological stability throughout imaging. Hypercapnia was induced by adding 2–3% CO_2 to the inhaled air mixture. A few minutes were allowed before blood gases were sampled again to confirm equilibration under hypercapnic conditions. After the last image during the hypercapnia was acquired, the last blood sample was drawn to verify the preservation of the hypercapnic condition. Typical experimental duration was 40 min (20 min for each of normo- and hypercapnia). The trials in which either normocapnia or hypercapnia could not be maintained were excluded from the analysis.