Pial arteriole dilation during somatosensory stimulation is not mediated by an increase in CSF metabolites

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Received 26 February 2001; accepted in final form 6 November 2001

Ngai, Al C., and H. Richard Winn. Pial arteriole dilation during somatosensory stimulation is not mediated by an increase in CSF metabolites. Am J Physiol Heart Circ Physiol 282: H902–H907, 2002; 10.1152/ajpheart.00128.2001.—Pial arterioles supplying the hindlimb somatosensory cortex dilate in response to contralateral sciatic nerve stimulation. The mechanism of this pial vasodilation is not well understood. One possibility is that vasoactive metabolites released during brain activation diffuse to subarachnoid cerebrospinal fluid (CSF) to dilate pial vessels. To test this hypothesis, we implanted closed cranial windows in rats and measured pial arteriolar dilation to sciatic nerve stimulation during constant rate superfusion of the pial surface with artificial CSF. We reason that flushing the pial surface with CSF should quickly dissipate vasoactive substances and prevent these substances from dilating pial arterioles. CSF flow (1 and 1.5 ml/min) significantly reduced pial arteriole dilation induced by 5% CO2 inhalation, but the same flow rates did not affect dilator responses to sciatic nerve stimulation. We conclude that brain-to-CSF diffusion of vasoactive metabolites does not play a significant role in the dilation of pial arterioles during somatosensory activity.

CEREBRAL ACTIVATION by sensory stimulation is accompanied by adjustments of vascular resistance and blood flow in the activated region. For example, we (13) have shown that pial arterioles supplying the hindlimb somatosensory cortex dilate in response to contralateral sciatic nerve stimulation. The mechanism of this pial vasodilation remains unclear. One possibility is that neuronal activity evoked by sensory stimulation causes the release of metabolites, which may diffuse to subarachnoid cerebrospinal fluid (CSF) to dilate pial arterioles. Putative vasoactive metabolites released during cortical synaptic activity include adenosine, H+, K+, and nitric oxide (1, 6). A variation of this diffusion mechanism is an arteriolar-venular countercurrent scheme (5) involving 1) the release of a vasodilator substance in the brain parenchyma and 2) the transport of the vasoactive substance by the draining venules to the pial surface.

METHODS

Experimental protocols in the present study were approved by the Animal Care Committee of the University of Washington. Adult male Sprague-Dawley rats weighing between 350 and 400 g were initially anesthetized with 2% halothane. The right femoral artery was exposed and cannulated for arterial blood pressure recording and for measurement of blood gas tension. The right femoral vein was cannulated for drug administration. The rats were tracheostomized, paralyzed with tubocurarine chloride (1 mg·kg−1, h−1 iv), and mechanically ventilated with a mixture of air and oxygen to achieve physiological arterial blood levels of pH, arterial Po2 (PaO2), and arterial Pco2 (PaCO2). Halothane anesthesia was gradually discontinued and replaced by α-chloralose and urethane (initial doses of 50 and 500 mg/kg ip, respectively, followed by maintenance doses of one-fifth the initial dose per hour). In all experiments, the time between the beginning of data collection and chloralose injection exceeded 2 h. An adequate level of anesthesia was indicated by stable and physiological levels of heart rate and blood pressure and by the lack of autonomic (cardiovascular) responses to surgical trauma and to somatosensory stimulation. End-tidal CO2 was continuously monitored, and rectal temperature was maintained at 37.6°C.

The animals were secured in a stereotaxic frame. The skull was exposed by a longitudinal incision from the occiput to the forehead. Dental acrylic was used to build a ridge around the cranial opening, and a closed cranial window was prepared as previously described (10). Polymethylene tubes (PE-50) were embedded in dental acrylic for the perfusion and drainage of artificial CSF. Intracranial pressure (IPC) was monitored via a third polymethylene tube. The composition of the artificial CSF was as follows: 156.5 mM Na+, 3.0 mM K+, 1.3 mM Ca2+, 0.7 mM Mg2+, 24.6 mM HCO3−, 66.5 mg/dl dextrose, and 40.2 mg/dl urea. The fluid was equilibrated with a gas mixture (5) involving

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mixture of 10% O₂-6% CO₂-84% N₂ and warmed to 37°C. The pH, $P_{CO_2}$, and $P_{O_2}$ after equilibration were 7.34–7.36, 36–38 mmHg, and 75–80 mmHg, respectively; osmolarity was 298 mosM. The dura-arachnoid membrane was incised. A microscope (Nikon) with long working distance objectives was used to observe the pial circulation, which was illuminated with a fiberoptic light fitted with heat filters.

The contralateral sciatic nerve was dissected free and cut proximal to the bifurcation into the tibial and peroneal nerves. The proximal end was placed on a pair of stimulating electrodes and bathed in a pool of warm mineral oil. The sciatic nerve was stimulated with rectangular pulses (0.5 ms) for 20 s. Stimulus intensity was adjusted to be at twitch threshold (~0.2 V), and blood pressure was not affected by stimulation. Both stimulus intensity and duration were kept constant during each experiment.

After completion of the preparation procedure, the animals were allowed to stabilize for ~20 min. Portions of the middle cerebral artery and vein supplying the parietal cortex were displayed on a video monitor by means of a videocamera. Vessel diameter was measured with a dimensional analyzer (IPM; San Diego, CA), which tracked the width of the red blood cell columns on-line.

The dilation response of pial arterioles to sciatic nerve stimulation was determined in animals under no-flow conditions with stationary CSF and 2) while superfusing the space under the cranial window with CSF at constant flow rates of 0.5, 1.0, or 1.5 ml/min. Higher flow rates inevitably caused rapid rises in ICP and/or leakage and, therefore, were not studied. At the onset of superfusion, the outflow tube was gradually lowered to maintain ICP at ~4 mmHg by adjusting the height of the outflow tube relative to the cranial window. A constant flow Harvard infusion pump was used to deliver pregassed and warmed CSF to the cranial window. The steady flow kept the vessel image in focus, thus facilitating continuous monitoring of vessel diameter during superfusion. Loss of gases from the CSF were minimized 1) by using gastight glass syringes, 2) by shortening the tubing length between the pump and the inflow tube, and 3) by using PharMed and metal tubings wherever possible. In addition, the CSF was passed through a jacket containing circulating water warmed to 38°C before it entered the cranial window. With such a setup, there was no appreciable change in CSF temperature with variations in flow rate.

In another series of experiments, the dilation responses to hypercapnia were recorded both during no-flow conditions and during constant CSF flow. Acute hypercapnia was induced by connecting the respirator to a gas mixture of 5% CO₂-40% O₂-55% N₂ for up to 3 min, after which normoxic ventilation was restored.

Arterial blood pressure, ICP, end-tidal CO₂, and vessel diameter were digitized and recorded on a PC computer using Axotape software (Axon Instruments; Foster City, CA). In addition, these signals were recorded continuously on a chart recorder. All data are expressed as means ± SE. Only one vessel was studied in each animal. Average responses to sciatic nerve stimulation were expressed as percent change from baseline (before stimulation) values. $CO_2$ reactivity was expressed as the percent increase in vessel diameter from baseline per mmHg increase in $P_{CO_2}$ using the average vessel diameter in the last 20-s period of hypercapnic gas inhalation for this calculation. Mean values were compared using Student’s $t$-tests. A $P$ value of <0.05 was considered significant.

RESULTS

The average arterial blood pressure (mean arterial blood pressure [MABP]) and blood gas values of all animals ($n = 28$) studied were as follows: MABP = 114 ± 6 mmHg, pH = 7.39 ± 0.02; $P_{CO_2} = 35.5 ± 1.1$ mmHg, and $P_{O_2} = 120.2 ± 5.2$ mmHg. Except during acute hypercapnia, these values were maintained stable throughout each experiment. Figure 1 depicts the typical response pattern of pial arterioles during sciatic nerve stimulation. The change in diameter exhibited a consistent profile: an initial brief (<2 s) latent period, followed by a rapid rise to a peak diameter, which subsequently declined to a plateau dilation that lasted for the remainder of the stimulation period. After the cessation of stimulation, diameter gradually returned to prestimulation values. There was no significant change in blood pressure during stimulation.

**Fig. 1.** Recordings from 1 experiment showing changes in diameter of a pial arteriole before (A) and during (B) superfusion of the pial surface at a rate of 1.0 ml/min. “On” and “off” indicate the onset and offset of sciatic nerve stimulation. MABP, mean arterial blood pressure.
As illustrated in Fig. 1, moving CSF (1 ml/min) had little effect on the dilator response to sciatic nerve stimulation (Fig. 1). Pooled data (Fig. 2) confirmed that neither the peak nor the plateau dilation responses were significantly affected by flushing the pial surface at flow rates up to 1.5 ml/min. Flowing CSF also did not affect baseline vessel diameter: resting diameters were 37 ± 2 vs. 38 ± 3 μm during flow at 0.5 ml/min, 44 ± 3 vs. 42 ± 3 μm during flow at 1 ml/min, and 42 ± 4 vs. 43 ± 4 μm during flow at 1.5 ml/min. We also characterized the temporal dynamics of the pial arteriolar response by measuring 1) the time between stimulus onset and peak dilation and 2) the time for the vessel to restore resting diameter after the end of stimulation. As shown in Fig. 3, CSF superfusion had no significant impact on these temporal parameters.

Figure 4 shows the time course of pial arteriole diameter changes during induction of hypercapnia by 5% CO₂ inhalation. Approximately 20 s after the abrupt rise in end-tidal CO₂, arteriole diameter gradually increased to steady values toward the end of the inhalation period. This increase in vessel diameter was curtailed by CSF superfusion at 1 ml/min. There was no appreciable change in mean blood pressure. Pooled data (Fig. 5) revealed that a flow rate of 0.5 ml/min did not significantly affect CO₂-induced dilation, whereas superfusion at the higher flow rates of 1 ml/min (n = 8) and 1.5 ml/min (n = 4) significantly reduced the hypercapnic dilation response. However, the 1.5 ml/min flow rate did not cause further suppression of vasodilation compared with flow at 1.0 ml/min. Similar results are seen by calculating CO₂ reactivity (Table 1). Table 1 also summarizes blood pressure and blood gas values before and during hypercapnia. As in the sciatic nerve stimulation studies, flowing CSF did not significantly affect baseline arteriole diameter (Table 1).
We also determined whether repeated episodes of CO₂ inhalation might lead to degraded dilation responses. CO₂-induced dilation responses of pial arterioles were determined sequentially in stationary CSF (control), during CSF flow (1 ml/min), and again in stationary CSF. Although CSF flow diminished the response of pial arterioles to hypercapnia (Fig. 6), dilation was restored to control values by stopping superfusion, showing that the reduction of hypercapnic vasodilation by flowing CSF was not due to deterioration of vascular reactivity.

DISCUSSION

We have shown that rapid superfusion of the surface of the brain did not affect pial arteriole dilation during somatosensory stimulation but may significantly attenuate the dilation response to hypercapnia. Thus pial arteriolar dilation during somatosensory stimulation is unlikely to be mediated by the diffusion of vasoactive metabolites from the brain parenchyma to the pial surface. In addition, the lack of effect of superfusion on resting diameter suggests that pial arteriolar resting tone was not influenced by CSF metabolites.

To our knowledge, this is the first study to examine the effect of CSF flow on pial arteriole dilation during somatosensory stimulation. A previous study (18) in rabbits determined the effect of cortical spreading depression on pial arteriole diameter before and during perfusion of the space under a closed cranial window. As in the present study, CSF flow had no effect on the pial hyperemic response to brain stimulation. In contrast, flushing the brain surface at 1 and 1.5 ml/min significantly curtailed the dilation response to hypercapnia. Previous investigators have also reported similar attenuation of hypercapnic dilation of pial arterioles by flowing CSF. Kontos et al. (9) reported that

![Graph showing dilation response to hypercapnia](image)

**Fig. 4.** Recordings from 1 experiment showing the effects of 5% CO₂ inhalation on the diameter of a pial arteriole before (A) and during (B) superfusion at 1 ml/min. Superfusion reduced hypercapnic dilation of the pial arteriole. MABP was not significantly affected by CO₂ inhalation.

![Graph showing pooled data](image)

**Fig. 5.** Pooled data. A CSF flow rate of 0.5 ml/min (left) did not affect vasodilation during 5% CO₂ inhalation, whereas flow rates of 1 ml/min (middle) and 1.5 ml/min (right) significantly suppressed hypercapnic vasodilation. Hypercapnic vasodilation (in %) = CO₂-induced increase in vessel diameter/baseline diameter × 100%. Values are means ± SE. *P < 0.05 vs. no flow.
cat pial arteriole dilation during inhalation of a gas mixture containing 7% CO₂ was completely abolished by flushing the brain surface with CSF containing no CO₂. In rabbits (18), cortical surface superfusion reduced the dilation of pial arterioles during inhalation of 10% CO₂ gas by ~60%. CO₂ reactivity in the present study was reduced by ~30% at a CSF flow of 1 ml/min, but because PaCO₂ values were not given in the rabbit study (18), these data cannot be directly compared with those from the present study.

Sensitivity to changes in PaCO₂ is a characteristic of the cerebral vasculature. An increase in arterial CO₂ tension leads to a corresponding rise in perivascular CO₂, due to local diffusion of CO₂ from the blood compartment. The subsequent decline in extracellular pH is pivotal for the dilation of pial vessels during hypercapnia (9). Rapid flushing of the pial surface with CSF could thus attenuate this dilation response, either by reducing local CO₂ tension or by dissipating perivascular protons. On the other hand, a component of this hypercapnic dilation response may not be sensitive to CSF superfusion. Because pial arterioles are partially invaginated in brain parenchyma (11, 12), CO₂ may diffuse not only into CSF but also into parenchyma in contact with these vessels. Pial arterioles may also be exposed to CO₂ released into brain tissue by intracortical arterioles and capillaries. Moreover, propagated vasomotor responses to local pH changes, initiated in parenchymal arterioles (2), may contribute to the pial vasodilator response. Thus CSF flow may not totally abolish the dilation response of pial arterioles during hypercapnia. In the present study, a flow rate of 1.0 ml/min attenuated pial vasodilation by clearing perivascular CO₂ from the pial surface. Higher flow rates (1.5 ml/min), however, did not cause further diminution of the pial dilation response. The residual dilation probably represents the component of the total dilation response that is unaffected by topical CSF flow.

The enhanced metabolism that accompanies cortical activation likely leads to the release of vasodilator by-products (6). Because of cellular uptake and biochemical inactivation, the movement of these substances is highly restricted in the brain parenchyma (3). However, if generated at a site near the brain surface, these metabolites may rapidly cross the pia-glial membrane to accumulate in CSF around pial arterioles. The diffusion distances are likely to be short: the pia-glial layer is only several micrometers thick (11) and is readily permeable to a wide variety of drugs and metabolites (3). Wei and Kontos (19) have calculated the time needed for a given concentration of solutes to diffuse various distances from the brain parenchyma. For substances such as CO₂, sucrose, and potassium ions, the time needed to achieve one-half their initial concentration after traversing a distance of 10 μm ranges from ~0.1 to 0.3 s (19). These values suggest that...
metabolites generated at the cortical surface not only may deliver vasodilators directly to pial arterioles but also may quickly spread to CSF, where they may dilate pial arterioles during somatosensory stimulation.

We reasoned that a CSF flow rate of 1 ml/min should be fast enough to dissipate metabolites at the pial surface and prevent these substances from dilating pial arterioles. The dilation response to sciatric nerve stimulation has a rise time (time to peak) of 7–9 s. Because the volume of the cranial window in this study is 0.06–0.08 ml (10), a flow rate of 1 ml/min could completely replace subwindow fluid once every 4–5 s, or 12–15 times/min. If pial arteriole dilation to somatosensory stimulation were primarily mediated by CSF metabolites, flushing the pial surface at 1 ml/min should have a marked impact on such a vascular response. In contrast, we found that superfusion had no effect on the dilation response during sciatic nerve stimulation even at a high flow rate of 1.5 ml/min. We therefore surmise that the brain-to-CSF diffusion of metabolites does not play an important role in the dilation response, likely because little metabolites reach the cortical surface as they spread from their local sites of generation.

As in hypercapnic vasodilation, the response of pial arterioles during cortical activation may occur by mechanisms that are insensitive to CSF superfusion. The intraparenchymal diffusion of metabolites released during neural activity is unlikely to be affected by topical CSF flow but may initiate vasomotor signals in nearby parenchymal vessels that propagate upstream, via gap junctions, to dilate surface vessels (2, 17). Alternatively, a pressure drop due to parenchymal vasodilation may induce the myogenic relaxation (4, 16) of pial arterioles. Another flow-insensitive mechanism involves intrinsic neurons, which can provide almost instantaneous communication between the parenchyma and pial arterioles. The presence of such vasodilator neurons has, however, never been convincingly demonstrated. Moreover, alterations of sympathetic and parasympathetic cholinergic mechanisms by superior cervical ganglionectomy, atropine application, and lesioning of cholinergic input did not affect the pial dilator response to sciatic nerve stimulation (7). Pial arterioles may also dilate by a shear stress-dependent mechanism (4, 14). Synaptic activity evoked by somatosensory stimulation may initially cause the dilation of parenchymal arterioles, leading to an increase in blood flow. This could then induce an increase in shear stress in pial arterioles, resulting in “flow-mediated dilation” (4, 14). However, the simultaneous measurement of flow velocity and pial arteriole diameter (15) revealed no significant change in wall shear rate during sciatic nerve stimulation. Thus the dilator response of pial arterioles to sciatic nerve stimulation is unlikely to be related to changes in blood flow velocity.

Besides the direct brain-to-pial surface diffusion, vasoactive substances released during cortical activity may diffuse into the microvasculature in the vicinity of the active neurons. These substances may subsequently be carried in venous blood to pial venules, where they may be released to act on arterioles. Such a venuloarterial countercurrent mechanism (5) requires the diffusion of dilator substances from venules to arterioles. The present study shows that moving CSF, which would dilute the diffused substance, had no effect on the vascular response to stimulation. Thus communication between venules and arterioles is unlikely to play an important role in the pial functional hyperemic response.

This study was supported by National Institute of Neurological Disorders and Stroke Grants NS-21076 and NS-07144.

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