Hirosaki Med. J. 61(Suppl.) : S181—S186, 2010

CEREBROVASCULAR DYNAMICS IN RESPONSE TO NEURAL STIMULATION

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Abstract Cerebral blood flow is tightly regulated, and local metabolic demands are met by adjustments to the regional density of microvascular networks and by temporal and spatial changes in microvascular blood flow. Cerebral blood flow regulation may involve communication between and across the vascular cells and neural or glial cells in either rapid or slow conduits. In the present study, we report distinct differences between the dynamic reactions of cerebral arterial networks in cortical surface and in intracortical tissue regions in response to sensory stimulation. Using confocal and multi-photon excitation laser scanning fluorescence microscopy, we imaged the cortical surface and subsurface vascular networks in the somatosensory cortex of isoflurane-anesthetized rats. Changes in lumen diameter were imaged at a rate of 13 frames per second with a field of view of 512 by 512 pixels. We consistently observed a stimulus-dependent increase in the lumen diameter of arterial networks in both cortical surface and subsurface regions. The onset time of vasodilation was observed to be ~0.8 sec for the subsurface arterioles (<40 μm), which was significantly shorter than the ~1.1 sec vasodilation onset time of the surface arteries (20-120 μm). The peak dilation accounted for 10% of the pre-stimulus baseline diameter. Further, the propagation of surface arterial vasodilation increased in a stimulus-dependent manner. The results indicate that global vasodilation of upstream parent arteries may be necessary to prevent "blood steal" by inactive regions nearby. Further studies are needed to elucidate the physiological mechanisms underlying the propagation of vasodilation induced by neural stimulation.

Hirosaki Med. J. 61, Supplement : S181—S186, 2010

Key words: two-photon microscopy; in vivo optical imaging; neurovascular coupling; functional brain mapping

Introduction

Blood flow circulation in the brain must be well coordinated in order to meet the variable energy demands induced by regional changes in neural activity. Long-term regulation of blood flow includes the formation of microvascular networks of the optimal density to minimize a hypoxic region in which tissue oxygen tension is low[1-4]. Short-term regulation of blood flow includes the increase of microvascular blood flow to active regions of the brain[5-9]. The close correlation of neurovascular compartments suggest that the brain microvascular system is tightly linked to the activity of local neural circuits. However, it remains unclear how vascular cells communicate with neural cells to dynamically transform neural signals into changes in blood flow.

The vasodilatory signal that is locally delivered is known to be conducted along the arterial networks via cell-to-cell communication within and across the microvascular smooth muscle cells and endothelium cells[10,11]. Using isolated cerebral arterioles, Horiuchi et al.[12] showed that local application of potassium ions causes vasodilation at a region farther than 1 mm from the stimulation site. Since this remote vasodilation was blocked by endothelial impairment, the

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authors suggest that the vasodilatory signal spreads along the endothelial cell layer. Conducted vasodilation that occurs at a distance from the stimulated neurons was also reported in cerebellar cortex *in vivo*[13]. These authors showed that direct electrical stimulation produces an increase in the vascular diameter of upstream parent arterioles; this increase is independent of spreading neural activity. Another study has also reported upstream arterial vasodilation due to signal conduction via the glia limitans in rat cerebral cortex *in vivo*[14]. Here, neuronal activation was induced by seizure and sciatic nerve stimulation. These studies suggest that the coordination of local and remote vasodilation is a key mechanism underlying spatiotemporal adjustment of microvascular blood flow to meet local demands. However, the spatiotemporal relationships of the vascular response at the proximal microvascular site where neural processing is ongoing in intracortical tissue layers and at distal upstream sites where is located on the cortical surface have not yet been determined.

In the present study, the temporal dynamics of the vascular response to neural stimulation was characterized in the cortical surface and in intracortical regions of the anesthetized rat somatosensory cortex. The cortical surface vascular networks and intracortical vessels were visualized with confocal and multi-photon fluorescence microscope systems, respectively. The dynamic changes induced by electrical forepaw stimulation were determined using time-lapse imaging at 13 frames per second, and surface and subsurface vascular reactions were compared. We also tested the activity-dependence of the vascular response by applying multiple stimulations (1, 2, 3, 4, 5 and 10 pulses). Changes in the lumen diameter induced by stimulation were determined by subtracting the images obtained during activation from images taken during the baseline period. Our results show that there are distinct differences in the dynamic responses of arterial networks in cortical surface and intracortical regions.

Materials and Methods

Animal preparation

All animal experiment protocols were approved by the Animal Experiment Committee of the National Institute of Radiological Sciences. A total of ten Sprague-Dawley rats (210-390 g) were used for the experiments. Five animals were used for the cortical surface vascular experiments, and six animals were used for the intracortical vascular experiments; one animal was used in both experiments. Animals were anesthetized with ~5% isoflurane for induction, ~2% isoflurane for surgery, and ~1.4% isoflurane for the experiments. The animals were ventilated and physiologic parameters (e.g., end-tidal CO_2 gas and isoflurane concentrations, heart rate, systemic arterial blood pressure, and blood gases including pH, $paCO_2$, paO_2, Glucose, Na^+, K^+, Ca^{2+}, and hematocrit levels) were monitored, and respiratory rate and minute ventilation volume were adjusted as needed. Rectal temperature was maintained at 37.0 ± 0.2 °C. A portion of the left skull (3 mm × 3 mm) over the somatosensory area was removed while keeping the dura intact. The opened area was filled with physiologic saline solution (37 °C). Two needles were inserted under the skin between digits two and four of the right forepaw, and electrical pulse stimulation was induced[15]. The electrical pulses were of 1.0 ms duration, 1.7 mA current, and either 83 ms or 167 ms onset-to-onset intervals.

In vivo vascular imaging

Intravenous injection of fluorescent quantum dots (1 μM in buffered solution, Invitrogen) was performed to fluorescently mark blood plasma. The vascular image was obtained using a confocal microscope system with multi-photon excitation (TCS SP5, Leica Microsystems). The vascular structure of the cortical surface was visualized on

the confocal system using a 488-nm argon laser and a 5× objective lens (0.12 NA), whereas the vascular structure in the subsurface cortex was visualized using a multi-photon excitation system with a 20x water-immersion lens (1.0 NA, Leica Microsystems), and with a Ti:Sapphire laser (Mai-Tai, Spectra-Physics) adjusted for excitation at 900 nm. The standard image (512 × 512 pixels) was obtained with an in-plane (x-y direction) resolution of 3.6 μm (5× objective lens) or 0.89 μm (20× objective lens).

Experimental protocol

Vascular images of the cortical surface at a single fixed location (FOV: 1.8 mm × 1.8 mm) were obtained by time-lapse imaging on the confocal system while multiple electrical stimulations (1, 2, 3, 4, 5, and 10 pulses with 83-ms intervals, and 18 pulses with 167-ms intervals) were applied (N = 5 animals). Time-lapse intracortical vascular images were acquired using the multi-photon system (N = 6 animals). Images were captured at a fixed depth within a range of 0.05 to 0.6 mm from the cortical surface. One to fourteen locations were imaged in each animal while electrical stimulation (18 pulses with 167 ms intervals for a total duration of 3 sec) was applied. We identified the vessel perpendicular to the cortical surface by visually tracking it from the surface vascular networks to deep cortical layers. Depending on the width of the cross-section (i.e., the lumen diameter) of the target vessels, the field of view (0.46 mm × 0.46 mm as a standard) was adjusted up to two times using the instrumental zoom function. Arterial and venous vessels were distinguished based on their pulsation and branching geometry at the cortical surface. One scanning run consisted of a total of 256 frames at 13 frames per sec, and the stimulation was given after a 5 sec pre-stimulus baseline scan (65 frames). In each experiment, the scanning run was repeated 16 times with an inter-run interval of 33 sec and then averaged across all runs. The region of interest was selected for measurement of cross-sectional width of the target vessel and for integration of pixel intensity in the vessel area. Normalized intensity (ΔF/F) was then reported by dividing the integrated pixel intensities by the pixel intensities at the pre-stimulus baseline level. Onset time of the vascular response was determined by measuring the time when the pixel intensity surpassed the mean of the pre-stimulus baseline plus 2 standard deviations. The time to peak was also reported. The data are represented as means ± sd.

Results and Discussion

Stimulation-induced increases in the vascular lumen diameter were consistently observed on the arterial side in both cortical surface and subsurface regions. The arterioles in the subsurface region responded more quickly compared to the arterial networks on the cortical surface (Fig. 1). The onset time of vasodilation

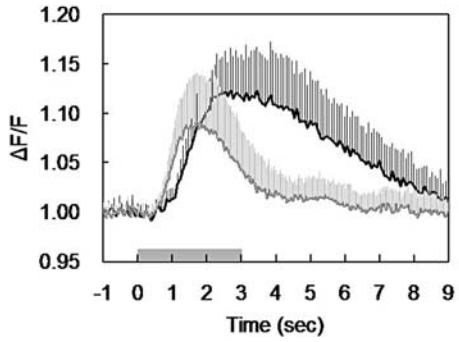

Figure 1 Temporal dynamics of vascular diameter changes induced by forepaw stimulation.

The change in vascular diameter was measured by calculating the relative changes in the pixel intensity (ΔF/F) of the vascular area from baseline (a mean of 1 sec pre-stimulation), while the forepaw stimulation was induced for 3 sec (18 pulses with 167 ms interval) from time 0 (gray bar). After the onset of stimulation, the subsurface cortical arterioles (<40 μm in diameter, gray) showed a rapid increase in cross-sectional area measured with pixel intensity changes. This signal increase was subsequently observed at the surface arteries and arterioles (20-120 μm in diameter, black). After the cessation of stimulation, we observed a sustained vascular response in the cortical surface arteries that was not observed in the subsurface arterioles. These results indicate that vasodilation induced by neural activity occurs first at the intracortical arterioles and then spreads into the upstream parent arteries.

was 0.8 ± 0.3 sec for the subsurface arterioles (<40 μm in diameter), which was significantly shorter than the 1.1 ± 0.2 sec vasodilation onset time of the surface arteries (20-120 μm in diameter). The time to peak of the vasodilation was also significantly different: 1.7 ± 0.4 sec vs. 3.0 ± 0.8 sec for subsurface arterioles vs. surface arteries. We performed the same set of experiments in our preliminary studies using laser-Doppler flowmetry (LDF) to measure blood flow responses. The results showed that blood flow dynamics were more similar to those of the surface arterial response. The close correspondence of the surface arterial response and red blood cell flow as measured with LDF was reported[16]. However, we observed a dissociation of temporal dynamics between the cortical surface and intracortical tissue arteries, indicating that separate regulatory mechanisms exist in the cortical surface and intracortical arterial networks. Since we observed a significantly faster response in the intracortical arterioles, the vasodilatory signal may propagate from the proximal intracortical regions (the main site of neural processing) to the distal upstream arteries located on the cortical surface.

As we increased the number of stimulus pulses, the response area of the surface arterial networks also increased. Typical results depicting activity-dependent vasodilation are shown in Figure 2. A mild stimulation (i.e., fewer number of pulses) induced a small area of

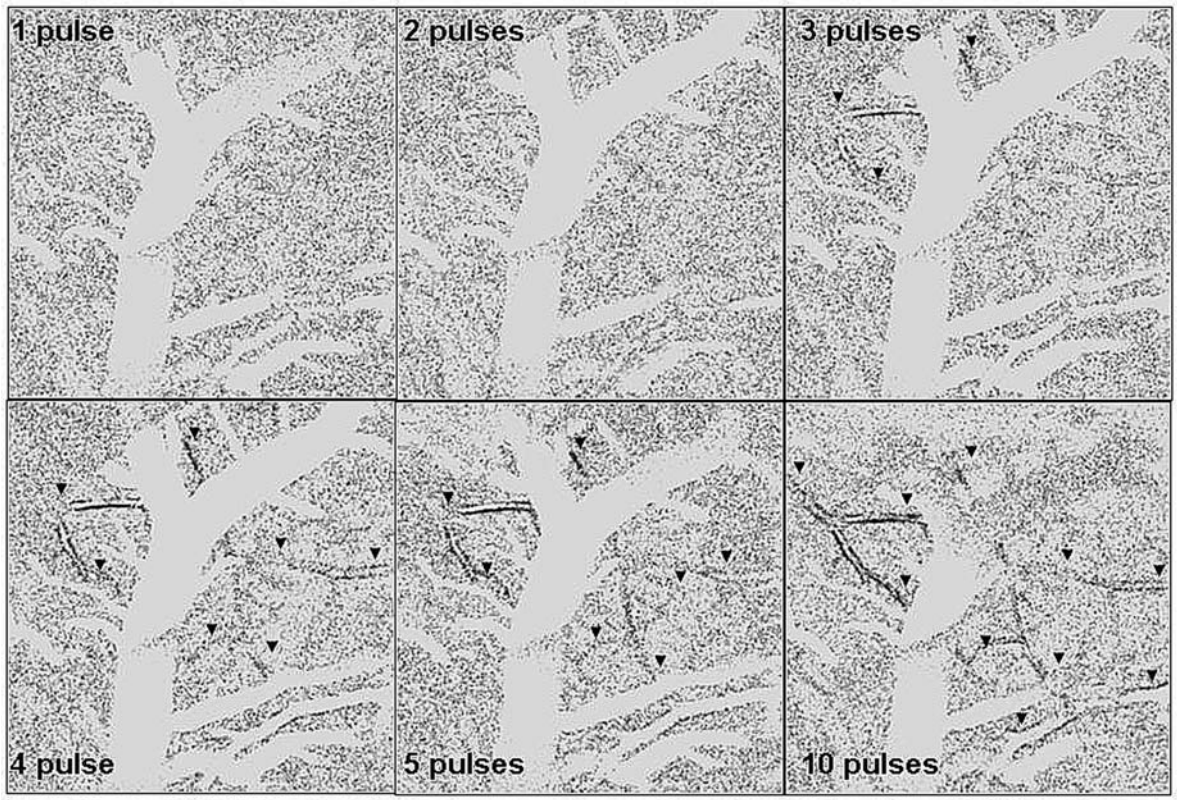

Figure 2 Activity-dependent propagation of vascular diameter changes induced by forepaw stimulation.
We stimulated the contralateral side of the forepaw using 1, 2, 3, 4, 5, and 10 electrical pulses and measured the cerebrovascular response in the somatosensory area. The location of the vascular diameter change (arrow head) was determined by subtracting the image taken during activation (a mean of 5 sec after the stimulation) from the baseline image (a mean of 5 sec pre-stimulation). The relative pixel intensity between the two images is represented by an 8-bit gray scale. Dark colors represent an increase in the intensity relative to baseline (i.e., an increase in lumen diameter due to stimulation). Note that activity-dependent increases in the area of the vascular response were observed, indicating that a vasodilatory signal spreads along the arterial networks in a neural activity-dependent manner. This stimulus dependence of the vascular response was observed consistently (N = 5 animals, FOV = 1.8 mm × 1.8 mm).

vasodilation, whereas a strong stimulation (i.e., greater number of pulses) induced a wide area of vasodilation. This spread of vasodilation was also observed outside of the receptive field of the forepaw area (assuming no detectable evoked activity), indicating that the vasodilatory signal propagates along the vessel trees. These results suggest that dilation of intracortical arterioles alone is not sufficient to produce adequate blood flow supply when local activity is elevated. Further studies are necessary to elucidate the mechanism of how upstream arterial vasodilation induced by neural stimulation is regulated.

Using the hydrogen clearance method[17] and autoradiography[18], it has been shown that the cerebral blood flow response to neural stimulation varies across cortical layers in the rodent somatosensory cortex. The layer-dependent differences in the temporal dynamics have also been demonstrated with magnetic resonance[19-21] and optical imaging[22]. In the present study, our results indicate that the vasodilatory signal propagates from the intracortical tissue to upstream parent arteries. However, the propagation speed was observed to be relatively fast (e.g., >~1 mm/sec) in our experimental conditions. Future studies are needed to assess the impact of the vasodilatory lag time on the dynamics of the hemodynamic signals observed with other depth-resolved hemodynamic imaging tools. The propagation speed attributed to small nerve fibers has been reported to be one order of magnitude slower (0.2 mm/sec) than what we observed in the present study[23]. The observed propagation of vasodilatory signals could therefore be conducted by electrical signals or calcium waves along endothelium cells or smooth muscle cell layers[24, 25]. It is also possible that multiple signals and multiple systems (e.g., glial networks) may participate in this vascular cell communication.

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