Effect of Transient Forebrain Ischemia on Flavoprotein Autofluorescence and the Somatosensory Evoked Potential in the Rat

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Abstract In order to evaluate the effect of cerebral ischemia on the flavoprotein fluorescence (FPF), we compared the changes in the FPF and somatosensory evoked potential (SEP) during transient cerebral ischemia in the rat. We measured the FPF and SEP simultaneously via a cranial window made over the right sensorimotor cortex during the left median nerve stimulation in F344 rats. We compared change in FPF and SEP during cerebral ischemia for 60 min. The rCBF were rapidly recovered after reperfusion. However, the recovery rates of the FPF were significantly faster than those of the SEP after reperfusion. These findings indicate that activity-dependent changes of the FPF do not necessarily correlate with the electrical activity after transient cerebral ischemia.

1 Introduction

Recently, it has been demonstrated that the autofluorescence of flavoproteins is applicable for brain functional imaging in rats [7]. Flavoproteins are involved in the electron transfer system of mitochondria. Neuronal activity increases the intracellular Ca^{2+}, which converts the flavoprotein from the reduced form to the oxidized form. The oxidized flavoprotein emits green autofluorescence upon excitation with blue light. Studies on flavoprotein fluorescence (FPF) imaging have demonstrated that changes in the FPF in response to sensory stimulation are much faster and more localized compared to those in the cerebral vascular responses [7]. Furthermore, measurement of the FPF is more stable than that of the NADH fluorescence response [6]. FPF has been applied to functional imaging in the sensorimotor cortex [7], cerebellar cortex [6], auditory cortex [9], and visual cortex [10, 4] in rodents and cats.

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However, it is not yet clear how cerebral ischemia affects the fluorescence; previous functional studies using the FPF have been conducted under normal circulatory conditions [7, 6, 9]. This issue is important for application of the FPF to research on functional reorganization after neuronal injury such as ischemic stroke. In the present study, we evaluated the effect of transient cerebral ischemia on the activity-dependent changes of FPF in the rat. We compared the changes of the FPF and somatosensory evoked potential (SEP) induced by transient cerebral ischemia.

2 Methods

2.1 Experimental Protocol

We employed adult male Fischer 344 rats (250 and 300 g, $n = 10$) anesthetized with 1.5% halothane. The right femoral artery was cannulated for recording of the arterial blood pressure and sampling of blood gases. The bilateral common carotid arteries were exposed, and closed with a tourniquet for transient forebrain ischemia. The rats then were fixed in a stereotaxic frame, and the right parieto-temporal bone was thinned (5 × 5 mm) over the sensorimotor cortex. Transient forebrain ischemia was induced by occlusion of the bilateral common carotid arteries for 60 min. The rectal temperature was maintained at about 378C with an automatic heating pad. The arterial blood pressure (110 ± 8 mmHg, mean ± SD) remained stable throughout the experiments. The present animal experiments were performed carefully in accordance with the guiding principles for the care and use of laboratory animals approved by Nihon University School of Medicine.

2.2 Measurements of Flavoprotein Fluorescence

We recorded images of the green autofluorescence (barrier filter bandwidth, 500–550 nm) excited with blue light (excitation filter bandwidth, 450–490 nm) employing a cooled CCD camera system (AQUACOSMOS, Hamamatsu Photonics, Hamamatsu, Japan). The method of Britton Chance was referred to for measurement of the ratio of autofluorescence [3]. The left median nerve was stimulated with bipolar needle electrodes. The autofluorescence images (Fig. 1a) were analyzed by a pixel-by-pixel division to detect intrinsic signals, and the time course of the autofluorescence intensity was estimated (Fig. 1b). The autofluorescence responses reached a peak at 0.8–1.0 s after the stimulus onset, while the hemodynamic responses reached a peak at 2.0–2.5 s after the stimulus onset.

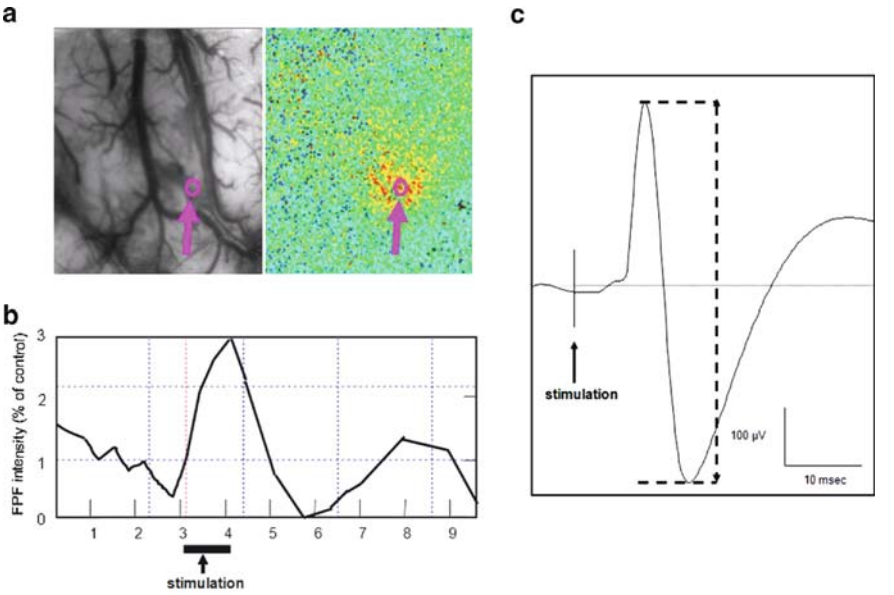

Fig. 1 a: Anatomical image of the recording area (*left*) and pseudo-color image of FPF changes during activation (*right*). b: Time course of the FPF intensity (*pink circle* in a) during activation. The ordinate indicates % change of FPF intensity. The thick horizontal bar indicates the period of electrical stimulation (1 s). c: Typical example of the SEP. The peak-to-peak amplitude was measured (*dotted lines*).

2.3 Measurements of SEP

We recorded the SEP with a Ag/AgCl ball electrode positioned at the forelimb area in the right sensorimotor cortex during left median nerve stimulation. A reference electrode was attached to the surrounding muscles and the ground lead was inserted in the right hind limb. The electrical stimulation was provided by a stimulator (SYNAX 2100, NEC), and 50 responses were averaged with a bandpass filter (10–1000 Hz). The amplitudes were measured peak-to-peak using the primary cortical response (Fig. 1c).

2.4 Data Analysis

Changes in SEP amplitude and FPF intensity were expressed as percentages of the responses under non-ischemic conditions (i.e. % SEP amplitude and % FPF intensity). Changes in regional cerebral blood flow (rCBF) caused by transient cerebral ischemia were measured with a laser-Doppler flowmeter (OMEGA-FLO FLO-N1, Omegawave). Changes in rCBF were expressed as a percentage of the non-ischemic baseline (i.e. % rCBF). Statistical analysis was performed

by means of an unpaired Student's *t*-test for comparisons involving the two ischemia models.

3 Results

Initially, we evaluated the frequency responses and intensity responses of the FPF and SEP under non-ischemic conditions ($n = 5$). Maximal SEP amplitude was observed at 0.5 Hz, and decreased with increase of the stimulus frequency. In contrast, the intensity of the FPF increased ofwociswithincrease

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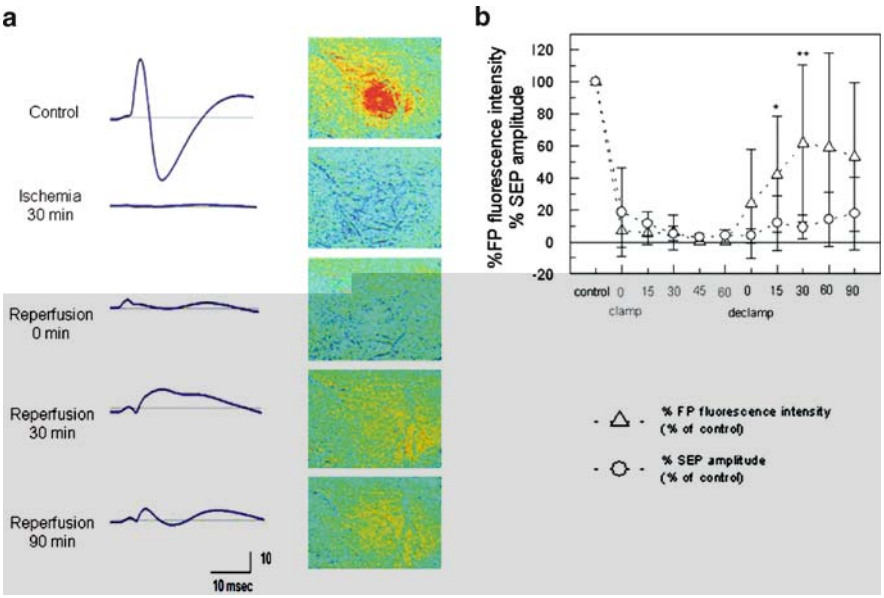

Fig. 3 a: Changes of SEP amplitude and FPF intensity during cerebral ischemia and after reperfusion in the 60-min ischemia model. b: There were significant differences in recovery rate between the SEP amplitude and FPF intensity at 15 and 30 min after reperfusion (*$p <$ 0.05, **$p < 0.01$)

4 Discussion

The present study demonstrated that the recovery rate of the SEP amplitude was significantly slower than that of the FPF after transient forebrain ischemia for 60 min. These findings indicate that transient cerebral ischemia affects the relation between activity-dependent changes of the FPF and the electrical activity.

Although the physiological mechanism of the dissociation in recovery rates between the FPF and SEP is not yet clear, FPF signal from astrocytes may play a role in the higher recovery rate of the FPF after reperfusion. Several reports have proposed that astrocytes play an important role in the active control of neuronal activity and synaptic neurotransmission [1, 5]. Astrocytes respond to neuronal activity with an increase of their internal Ca^{2+}, which triggers the release of chemical transmitters from the astrocytes themselves and, in turn, causes feedback regulation of the neuronal activity and synaptic strength. Such activity of astrocytes during neuronal activity could induce oxidation of flavoprotein in the electron transfer system, leading to emission of autofluorescence similar to that of neurons. Indeed, it has been demonstrated that the autofluorescence intensity was significantly decreased by the blocking of glial activity [2]. It should be noted that astrocytes are relatively resistant to ischemia as

compared to neurons [8]. These observations suggest that the FPF signal from astrocytes might be involved in the greater recovery of the FPF response after reperfusion in the 60-min ischemia model.

Conclusion: The present findings indicate that activity-dependent changes of the FPF do not necessarily correlate with the electrical activity after transient cerebral ischemia. The following mechanisms are suggested for the dissociation in recovery rates of the FPF and SEP after reperfusion. First, the FPF signal from astrocytes may contribute to the higher recovery rate of the FPF. Second, there are differences in ischemic tolerance between the cortical layers which generate FPF and SEP signals; the second and third layers which generate the FPF are more resistant to ischemia than the fourth layer which generates the SEP.

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