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BRAIN RESEARCH

Research Report

Simultaneous observation of superficial cortical and intracerebral microvessels in vivo during reperfusion after transient forebrain ischemia in rats using synchrotron radiation

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ABSTRACT

Using a newly developed angiography system that combines monochromatic synchrotron radiation (MSR) as an X-ray source with a high-definition camera or video system, we observed superficial cortical and intracerebral microvessels simultaneously in vivo during reperfusion after transient forebrain ischemia. Transient brain ischemia was induced by 10-min four-vessel occlusion in rats under general anesthesia. Angiographic images were then sequentially obtained at 3 frames/s. The detector features a 7-μm equivalent pixel size projected onto the input area and a 7 mm × 7 mm input field. Changes in the cerebral microvessels were observed before and 1, 5, 10, 15, 20 and 30 min after transient cerebral ischemia using the MSR angiography system. The calibers of the internal carotid artery (ICA), middle cerebral artery (MCA), and striate artery (SA) significantly increased 1 min after reperfusion, while the pial arteriole (PA) caliber significantly decreased (76% of base line). The MCA, PA and SA were significantly dilated 5 and 10 min after reperfusion. Although the caliber of the ICA significantly decreased after 30 min reperfusion compared with the basal value, the calibers of the other three vessels remained larger than the basal values throughout the experiment. Early venous filling was observed at 5 and 10 min after reperfusion. The MSR angiography system is useful for investigating morphological changes in both cortical and central branches of cerebral vessels in rats during reperfusion after cerebral ischemia.

1. Introduction

A rapid postischemic increase in cerebral blood flow relative to control values, i.e., hyperperfusion, has long been documented in animal stroke models, and hyperperfusion is the hallmark of efficient reopening of occluded arteries with subsequent reperfusion of the tissue (Marchal et al., 1999). Recognition of the actual behaviors of cerebral vessels during ischemia and reperfusion is the first step to understanding the pathophysiologic events of the brain suffering from ischemic injury (Ginsberg et al., 1997). Various methods have been used to achieve morphological observation of the cerebral vessels

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Table 1

	Before ischemia	During ischemia	After ischemia					
			1 min	5 min	10 min	15 min	20 min	30 min
MABP (mmHg)	102±11	37±20	99±11	109±12	108±14	107±12	107±11	104±12

Data are means±SD. MABP: mean arterial blood pressure.

during ischemia and reperfusion, with each having its merits and demerits. In animal models, pial microvessels are accessible by direct visualization using a cranial window technique equipped with confocal laser microscopy/fluorescence video imaging, but only to a depth of 250 μm from the cortical surface (Villringer et al., 1989). However, deeper microvascular beds such as those of the striate artery, which are not accessible with the cranial window technique, can be assessed using tracer perfusion techniques and reconstruction by computer-assisted static image analysis for study (del Zoppo et al., 1991). However, passage of all currently used tracers through the blood–brain barrier is limited, and as a consequence a nonlinear relationship between tracer uptake and true cerebral blood flow is observed. Accordingly, for study of ischemic injuries these techniques for direct visualization of microvascular beds in the brain allow only restricted observations of real-time events on the cortical surface and the basilar artery.

A new angiography system, consisting of monochromatic synchrotron radiation (MSR) as an X-ray source and a high-definition camera with a video system as a detector, has been developed in Hyogo, Japan (Umetani et al., 2002). This angiography system uses nearly parallel X-rays. Consequently, it can visualize microvessels with a caliber of 20–30 μm (Kidoguchi et al., 2006; Tokiya et al., 2004). Conventional X-ray imaging with an X-ray tube can depict only vessels with a caliber larger than 200 μm. This new system, therefore, offers higher resolution. In this study, we attempted in vivo simultaneous observation of pial arterioles and striate arteries in rats during reperfusion after transient forebrain ischemia using this MSR angiography system.

2. Results

2.1. *Physiological variables*

Six rats attained forebrain ischemia, but two did not. The mean arterial blood pressure (ABP) significantly increased by 135±18% during ischemia and then returned to its basal level immediately after reperfusion. There was no significant change in mean ABP while microangiography was performed (Table 1). The arterial blood gas levels were not significantly different 15 min before ischemia and 30 min after reperfusion (Table 2).

Table 2

	Before ischemia	After ischemia
pH	7.405±0.02	7.397±0.03
PaO_2, mmHg	89±4	87±4
$PaCO_2$, mmHg	38.1±2.7	39.1±3.4
Na, mmol/l	140±2	139±2
K, mmol/l	4.0±0.3	4.2±0.4
Hematocrit, %	46.2±1.5	43.2±1.9

Data are means±SD. PaO_2: arterial oxygen partial pressure; $PaCO_2$: arterial carbon dioxide partial pressure; Na: sodium concentration in serum; K: potassium concentration in serum.

2.2. *Basal conditions*

The diagram in Fig. 1 demonstrates the stereotactic co-ordinates of the image field of view in the rat brain. The MSR angiography system could detect a tungsten wire 100 μm in diameter and provided good visibility of the ICA, MCA, PA and SA under basal conditions (Fig. 2A). Each image in Figs. 2A–D depicts a temporal subtraction result for flat-field correction; the image taken before injection was subtracted from raw images taken after injection to eliminate the superimposed background structure. This system could also detect microvessels with a caliber of 20–30 μm. The mean ICA caliber was 401±44 μm, the mean MCA caliber was 179±21 μm, the mean PA caliber was 64±14 μm and the mean SA caliber was 53±18 μm.

2.3. *Reperfusion*

The ICA caliber significantly increased at 1 min after reperfusion, as shown in Fig. 2B, and those of the MCA and SA significantly increased at 1, 5 and 10 min after

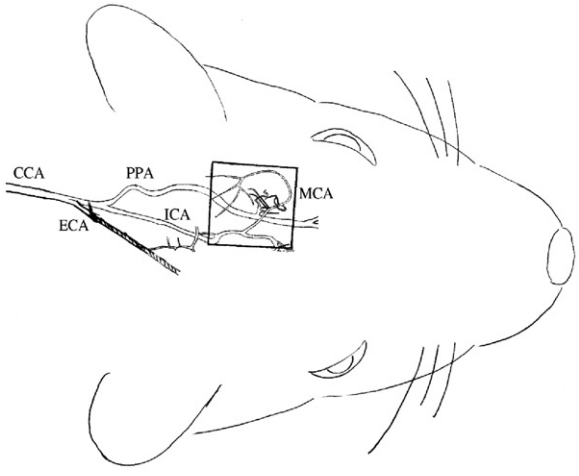

Fig. 1 – Diagram showing stereotactic co-ordinates of the image field of view in the rat brain. The quadrangular area of 7 mm×7 mm in the diagram is the area included in the photograph. CCA, common carotid artery; ECA, external carotid artery; PPA, pterygopalatine artery; ICA, internal carotid artery; MCA, middle cerebral artery.