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The Effect of Extracellular Glutamate Release on Repetitive Transient Ischemic Injury in Global Ischemia Model

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During operations, neurosurgeons usually perform multiple temporary occlusions of parental artery, possibly resulting in the neuronal damage. It is generally thought that neuronal damage by cerebral ischemia is associated with extracellular concentrations of the excitatory amino acids. In this study, we measured the dynamics of extracellular glutamate release in 11 vessel occlusion (VO) model to compare between single occlusion and repeated transient occlusions within short interval. Changes in cerebral blood flow were monitored by laser-Doppler flowmetry simultaneously with cortical glutamate level measured by amperometric biosensor. From real time monitoring of glutamate release in 11 VO model, the change of extracellular glutamate level in repeated transient occlusion group was smaller than that of single occlusion group, and the onset time of glutamate release in the second ischemic episode of repeated occlusion group was delayed compared to the first ischemic episode which was similar to that of single 10 min ischemic episode. These results suggested that repeated transient occlusion induces less glutamate release from neuronal cell than single occlusion, and the delayed onset time of glutamate release is attributed to endogeneous protective mechanism of ischemic tolerance.

Key Words: Repeated transient occlusion, Real time monitoring, Extracellular glutamate release

INTRODUCTION

In clinical conditions, surgeons in neurosurgical area usually perform the multiple temporary clippings of parental artery to avoid bleeding at the operative field. However, the effect of multiple temporary occlusions remains unclear. Since the brain is vulnerable to ischemic events, brief interruption of cerebral blood flow even for only five minutes can cause severe neuronal death in corresponding brain regions (Lee et al., 2000). However, most living organism invoke a self-protective mechanism and adapt or lessen upcoming lethal damage in order to survive in the adverse circumstances (Lin et al., 2008). This phenomenon is known as ischemic preconditioning or tolerance which was first demonstrated in the heart and later found in other organ systems including the brain. It is therefore necessary to confirm the beneficial or deteriorative effect by multiple temporary clipping.

The neuronal damage by cerebral ischemia is associated with extracellular concentrations of the excitatory amino acids (Zhao et al., 1997). Therefore a better understanding about excitotoxic process requires accurate assessment of the temporal changes in extracellular glutamate levels during and after various insults which are suspected to initiate this type of damage.

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In the present study, we measured the dynamics of extracellular glutamate release in 11 vessel occlusion (VO) model. And the changes of glutamate release in repeated 5 min ischemic episodes were compared with those of single 10 min ischemic event.

METHODS

11 VO model preparation

Six Sprague-Dawley (SD) rats (avg. =300 gm) were anesthetized with chloral hydrate (0.1 cc/100 mg with intraperitoneal injection). Eleven VO rats were prepared by dividing the omohyoid muscle to allow the larynx to be retracted, and exposing the ventral surface of the clivus following retraction of longus coli muscle. Three mm craniotomy was drilled through the clivus, centered just caudal to the basioccipital suture. After opening the dura and arachnoid, the distal basilar artery was coagulated just caudal to the superior cerebellar arteries, and divided. The pterygopalatine arteries were coagulated prior to the entrance into the tympanic bullae. Both occipital arteries and the superior thyroid arteries were identified, coagulated and transected. Snares were placed around the external carotid arteries (ECAs) between the occipital arteries prox-

ABBREVIATIONS: VO, vessel occlusion; ECAs, external carotid arteries; CCAs, common carotid arteries; EEG, electroencephalography; CBF, cerebral blood flow.

imally and the superior thyroid arteries distally, and snares were placed on the common carotid arteries (CCAs) (Fig. 1).

Real time monitoring of physiological parameters

Animals were placed in stereotaxic head holder (David Kopf Instrument, USA) for real-time glutamate monitoring. Eight burr holes were made; at 4 mm apart from the midline and 1 mm front of coronal suture, one for the glutamate probe insertion and another for brain temperature measurement. At 4 mm apart from the sagittal suture and 1mm behind of coronal suture, two burr holes were made for electroencephalography (EEG) electrodes. Two burr holes were made for CBF (cerebral blood flow) probe at both sides and at the 2 mm behind point of the EEG sites. And another two burr holes for EEG were made 2 mm behind of CBF probe at both sides. EEG electrodes were attached by 4 screws to acquire two channels of EEG signals which were amplified with 511 AC Amplifier (Grass product group, Astro-Med, Inc., USA), and the signals were then converted to digital information at 256 times per second by data acquisition system which was designed in our laboratory. EEG signal provided information on the electrical failure of the neuronal cell during ischemic episode. The probes for the CBF were attached at both hemispheres with BLF21D laser Doppler flowmetry (Transonic systems Inc., USA). The microdialysis electrode [Sycopel International Ltd. (Type: General 20-10-4-4, UK)] was filled with phosphate buffer saline (PBS) to electropolymerize the O-phenylenediamine on the platinum electrode at 0.65 V for 20 min with Sycopel BD2000 potentiostat (Sycopel INT. Ltd., UK). Fresh PBS containing glutamate oxidase was then perfused in the dialysis electrode at a flow rate of 0.5 μl/min. The dialysis electrode showed linear response in the concentration range of 50~450 μM standard glutamate solution with a sensitivity of 0.22 nA/μM (R^2, coefficient of regression of 0.998). After the sensor calibration procedure, the microdialysis electrode was inserted into motor cortex at coordinates A 1 : L 4 : V 4 mm (from bregma and the dura) through a small incision in the dura.

Single occlusion and repeated transient occlusion

After a control period for 10 min, a 10 min 11 VO cerebral ischemia was initiated by pulling the snares on the CCAs and the ECAs for single occlusion group. The snares were released and withdrawn after 10 min and reperfusion was performed for 1 h. For repeated transient occlusion group, the 11 VO models were exposed to 5 min episode of ischemia and reperfused for 20 min. Reperfusion in 20 min after 5 min occlusion was repeated twice.

Statistical analysis

Values were expressed as mean±SEM, obtained from six independent rats. Statistical analysis was carried out by independent t test.

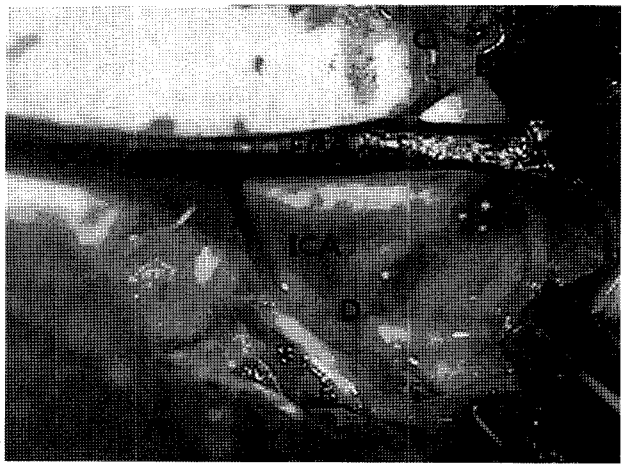

Fig. 1. Vascular anatomy of the neck dissection at left side. A, occipital artery; B, ascending pharyngeal artery; C, superior thyroid artery; D, pterygopalatine artery; ECA, external carotid artery; CCA, common carotid artery; ICA, internal carotid artery.

RESULTS

The pattern of ischemia-evoked response in 11 VO rat model was similar to both groups, as follows. CBF declined rapidly to near zero levels, concurrently with the development of a flat EEG signal. With a dialysis electrode, the elevated glutamate release began after the onset of ischemia and continued to rise throughout the ischemic period. A transient increase in glutamate was detected at first, however, the data showed a steady decline to pre-ischemic levels during reperfusion.

As shown in Fig. 2, the elevation of glutamate release in single 10 min occlusion group began at 113.16±35.79 sec after the onset of ischemic episode and continued to rise throughout the entire ischemic period. The glutamate level was then rapidly declined to pre-ischemic level during the reperfusion period. And the glutamate release started to change at 103.52±19.85 sec. in the first 5 min ischemic period of repeated transient occlusion group, as shown in Fig. 3. However, the elevation of glutamate release was delayed to 132.74±8.29 sec. in the second ischemic period of re-

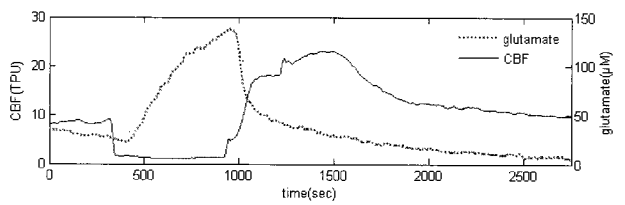

Fig. 2. Real-time measurement of glutamate release and CBF during single 10 min ischemic episode.

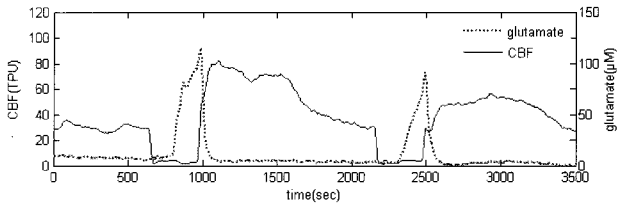

Fig. 3. Real-time measurement of glutamate release and CBF during repeated 5 min transient ischemic episode.

Table 1. The changes of glutamate concentration in 11 VO method during single 10 min occlusion and repeated 5 min occlusion

n=6	Single occlusion group (mean±S.E.M.)	Repeated transient occlusion group	
		First 5 min occlusion (mean±S.E.M.)	Second 5 min occlusion (mean±S.E.M.)
Onset time of glutamate release (sec)	113.16±13.07	108.01±11.73	132.74±3.03*
Maximum change of glutamate release (μM)	125.01±3.74	103.52±7.25**	88.49±4.77***
Total amount of glutamate release	38,390.44±2,252.86	11,021.56±2,596.68***	9,689.06±2,622.65***

*p<0.1, **p<0.05, ***p<0.001.

peated transient occlusion group.

As shown in Table 1, the maximum change of glutamate concentration was 125.01±10.25 μM in the single 10 min occlusion group during ischemic period, whereas the maximum change of glutamate concentration was 103.52±19.84 μM in the first ischemic period and 88.49±13.07 μM in the second ischemic period of repeated transient occlusion group. The total amount of glutamate release during ischemic episode was 38,390.44±6,169.70 in the single occlusion group and 20,710.62±18,277.71 (54% decline) in the repeated transient occlusion group. Table 1 shows that the analysis parameters for glutamate release were statistically significantly different between the two groups.

DISCUSSION

It is known that the excitotoxic hypothesis of ischemic neuronal death is based on neurotoxic effects of high extracellular concentrations of glutamate release as the result of enhanced excitatory synaptic activity (Katayama et al., 1991; Limbrick et al., 2003). This toxicity is largely related to calcium influx into affected nerve cells (Kristian and Siesjö, 1998).

It has been reported that brief episodes of ischemia induce neuronal tolerance to subsequent lethal ischemia (Dowden and Corbett, 1999). This phenomenon is defined as ischemic preconditioning or tolerance (Schaller and Graf, 2002). Many researchers reported that brief periods of global ischemia protect against subsequent prolonged global ischemia (Kitagawa et al., 1991; Kirino et al., 1991; Lin et al., 1992) Kitayama et al. (1991) reported that 2 min of ischemic preconditioning was followed by cerebral tolerance, and that a 24 h interval was necessary for induction of such a tolerance in gerbil model. Furthermore, as in myocardial cells, multiple brief ischemic episodes induced cerebral ischemic tolerance more potentially than a single episode. Nagata et al. (1993) showed by microdialysis sampling and HPLC analysis that glutamate release was subsequently decreased in repetitive ischemic events of Gerbil model, and Ueda et al. (1992) reported that the changes of extracellular glutamate concentrations showed no cumulative effect on repeated bilateral hemispheric ischemic events for 3 or 5 min in 4-vessel artery occlusion model.

On the other hand, however, Lin et al. (2008) reported that the glutamate release and ischemic brain tissue damage are increased after repeated episodes of global ischemia in a rat model, and Tomida et al. (1987) demonstrated that 3 separate, 5-min bilateral occlusions of the common carotid arteries induce neuronal damage. The variability of ischemic preconditioning effect has been suggested to be due to the difference of the duration of cerebral ischemia and the inter-ischemic interval.

Our present result showed that the change of extracellular glutamate release in multiple occlusion group was smaller than that of single occlusion group, and that the onset time of glutamate release in the second ischemic events in repeated occlusion group was more delayed than that of the first ischemic events which was similar to that of single 10 min ischemic episode. Shimazaki et al. (1998) reported that the intracellular calcium influx in ischemia-tolerant neurons is markedly inhibited after an anoxic-aglycemic episode, as compared with neurons in control animals, and Lin et al. (2008) showed that preconditioning with glutamate confers neuroprotection against subsequent oxygen-glucose deprivation in cultured cortical neurons. Furthermore, our results showed that glutamate release in multiple 5 min ischemic episodes was reduced subsequently by induced cerebral ischemic tolerance which inhibited intracellular calcium influx. The molecular mechanisms involved in ischemic tolerance are not yet completely understood. Nevertheless, it is highly likely that the onset of glutamate release was delayed by cellular defense function against ischemia which changed cell metabolism (Blanco et al., 2006). The further experimental efforts are needed to verify precise mechanism of cerebral ischemic tolerance by repeated transient occlusion.

Real time monitoring of glutamate release in 11 VO ischemia model demonstrated that multiple 5 min ischemic episodes showed beneficial effect on neuronal damage compared to the single 10 min ischemic episode, and that extracellular glutamate release by multiple transient injuries was suppressed by the ischemic tolerance which inhibited intracellular calcium influx. It is highly likely that the onset time of glutamate release was delayed by protective mechanism of ischemic tolerance. Therefore, multiple temporary clipping appears to be better than single long occlusion for clinical conditions in the neurosurgical area.

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