

THE DYNAMICS OF EXTRACELLULAR GLUTAMATE RELEASE ON REPETITIVE TRANSIENT ISCHEMIC INJURY IN DIABETIC RATS

^{1,2} Gija Lee, ² Sungwook Kang, ^{1,2} Samjin Choi, ³ Seokkeun Choi, ^{1,2} Jeonghoon Park, ^{1,2} Youngho Park, ^{1,2} Kyungsook Kim, ^{1,2,4} Bermseok Oh and ^{1,2,4} Hunkuk Park

¹Department of Biomedical Engineering, School of Medicine, ²Healthcare Industry Research Institute, ³Department of Neurosurgery, Kyung Hee University Medical Center, ⁴Program of Medical Engineering, Kyung Hee University, Seoul 130-702, Korea; email: sigmoidus@khu.ac.kr

INTRODUCTION

Stroke in patients complicated with diabetes mellitus (DM) is the major cause of mortality and disability [1]. Hyperglycemia makes worse the cerebral ischemic injury but the mechanisms remain unknown. The neuronal damage by cerebral ischemia is associated with extracellular concentrations of the excitatory amino acids [2]. 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.

In clinical conditions, neurosurgeons usually perform the multiple temporary clampings of parental artery to avoid bleeding at the operative field. However, the effect of multiple temporary occlusions remains unclear. Most living organism invoke a self-protective mechanism and adapt or lessen upcoming lethal damage in order to survive in the adverse circumstances. 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. In our previous study, we demonstrated that multiple 5 min ischemic episodes showed beneficial effect on neuronal damage compared to the single 10 min ischemic episode in global ischemia model [3].

In this study, we measured the dynamics of extracellular glutamate release to evaluate the effect of repetitive transient ischemic injury in diabetic rats.

METHODS

Male Sprague-Dawley rats (8 weeks old, 250g) were recruited. DM was induced by injection of streptozocin (STZ; 60 mg/kg; Sigma) into the tail veins. Forty eight hours after STZ injection, blood glucose was measured on a commercially available glucose meter (OneTough® Ultra™, LifeScan, USA) with blood samples taken from tail vein. Animals with blood glucose concentration >300mg/dl were selected as a DM model. The acute DM rats were used for experiments within 5 to 7 days after STZ injection. Four DM rats (DM group) and four normal rats (control group) were used for real-time measurement of glutamate release on repetitive ischemic injury. 11VO model of global ischemia was established as described earlier [4].

Animals were placed in stereotaxic head holder (David Kopf Instrument, USA) for real-time glutamate monitoring. 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). 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. Brain temperature was maintained at $37.5 \pm 1.0^\circ\text{C}$ by controller connected with epidural thermocouple.

After a control period for 10 min, 5 min 11 VO cerebral ischemia was initiated by pulling the snares on the CCAs and the ECAs in both groups. The snares were released and withdrawn after 5 min and reperfusion was performed for 20 min. Reperfusion in 20 min after 5 min occlusion was repeated three times.

RESULTS AND DISCUSSION

The change of extracellular glutamate level in DM group was larger than that of control. But extracellular glutamate release was gradually suppressed by repetitive transient ischemic injury in two groups. And the onset time of glutamate release in subsequent ischemic episodes was gradually delayed compared to the first ischemic episode in two groups. From real-time monitoring of glutamate release, we suggested that repeated transient occlusion showed beneficial effect on neuronal damage, though DM worsened the cerebral ischemic injury. Therefore, multiple temporary clipping appears to be better than single long occlusion for clinical conditions in the neurosurgical area.

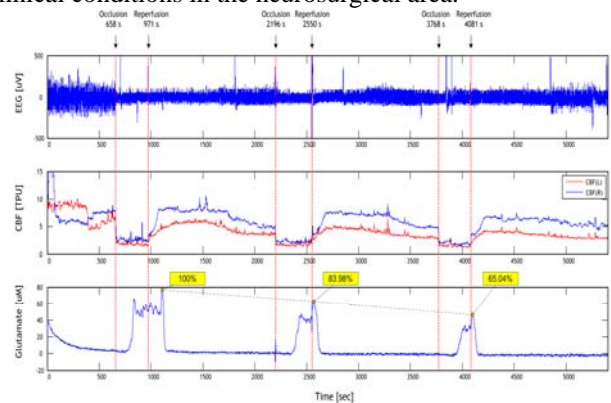


Figure 1: A Real time measurement of EEG, CBF and glutamate release on repetitive ischemic injury in diabetic rat.

ACKNOWLEDGEMENTS

This study was supported by the research fund from Seoul R&BD program (grant # CR070054).

REFERENCES

1. Kagansky N, et al., *Arch Rev.* **58**:1209-1212, 2001.
2. Zhao H, et al., *Neuroreport.* **8**: 2389-2393, 1997.
3. Lee GJ, et al., *Korean J Physiol Pharmacol.* **13**:23-26, 2009.
4. Choi SK, et al., *Korean J Integra Med.* **3**:11-16, 2008.