Effects of nasopharyngeal cooling on the dynamic changes in extracellular glutamate levels during anoxic depolarization and repolarization

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Introduction

The cascade of neuronal cell injury induced by ischemia is quite complicated.[1] Therefore, we focused on glutamate, which is the key substance at the start of the cascade. It is necessary to repolarize to recover neuronal cell function in vivo.[2, 3] We specifically focused on the glutamate levels, direct current (DC) amplitude, and cerebral blood flow (CBF) during repolarization. We also examined the effects of nasopharyngeal cooling on extracellular glutamate levels during repolarization.

Materials and Methods

All experiments were performed in accordance with the National Institutes of Health animal care guidelines and were approved by the Animal Research Control of Okayama University Graduate School of Medicine.

Animals: 20 male Sprague-Dawley (SD) rats (weight: 311±12.3 g)
The animals were randomly assigned to two groups
□ Normothermic group (n=10)
Brain- surface and rectal temperatures were maintained at 37.0 ±0.5℃.
□ Hypothermic group (n=10)
Brain Hypothermia (nasopharyngeal cooling) was initiated immediately after the onset of anoxic depolarization. The cerebral temperature was reduced to 31℃ at 4.9 ±1.0 min after the onset of nasopharyngeal cooling.
Anesthesia: Isoflurane
Respiration: Oral tracheal intubation and initiation of artificial ventilation
Temperature: Rectal and Epidural temperatures were monitored
Ischemia: Common carotid arterial occlusion and Exsanguination.
Statistical Analysis: All values are expressed as means ±SD. The data were analyzed by Student's t-tests and Mann-Whitney U-test.

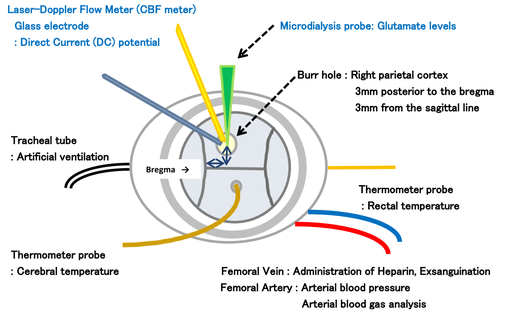
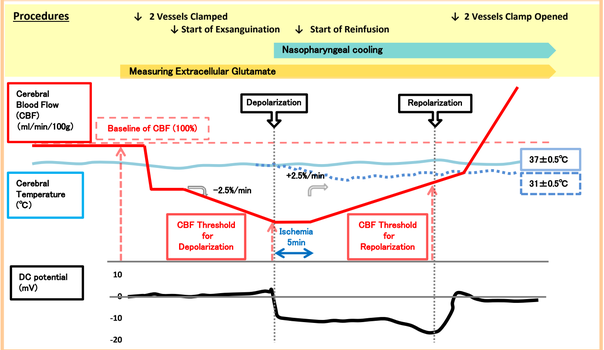


Figure 1 : A schematic drawing of the experiment
A microdialysis probe for measuring glutamate levels was inserted into the right parietal cortex adjacent to the direct current (DC) electrode and laser – Doppler flow probe.

Figure 2: How to Measure the Dynamic Changes in Extracellular Glutamate levels :
Bilateral Carotid Arterial Occlusion plus Exsanguination and Blood Reinfusion



After the preparation was completed, the baseline CBF was checked.

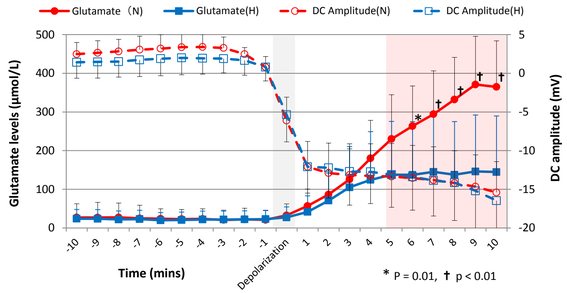
Following bilateral common carotid arterial occlusion, the CBF was continuously decreased by exsanguination at a speed of 2.5% of the baseline level every minute until depolarization occurred. After 5min of anoxic depolarization, CBF was restored at the same rate until a positive DC shift was observed.

In the hypothermic group, the brain temperature was decreased to 31℃ by initiating nasopharyngeal cooling immediately after the onset of depolarization.

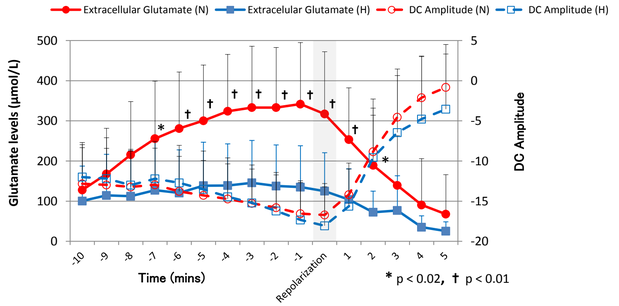
- CBF threshold for depolarization: CBF value at the sudden negative shift of DC potential.
- CBF threshold for repolarization: CBF value at the beginning of the increase of DC potential .

Results

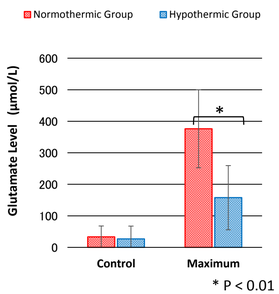
Graph1 . The effects of hypothermia on glutamate levels and DC amplitudes during depolarization



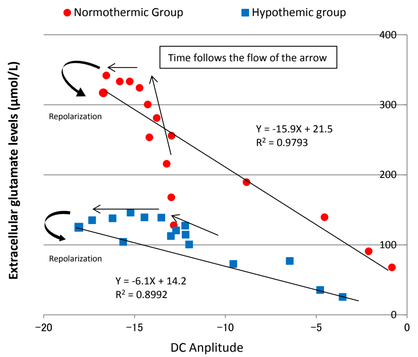
Graph 2. The dynamic changes in glutamate levels and DC amplitudes during repolarization



Graph 3. Maximum glutamate levels



Graph 4. Relationship of glutamate levels and DC amplitude during repolarization



Discussion

The mechanisms of the recovery of membrane potential in vivo

- ✓ Recovery of ion equilibrium
- ✓ Decrease in extracellular glutamate

Causes of glutamate decrease

- ✓ Washed away by CBF into blood
- ✓ The "reversed operation" of glutamate transporters stop
- ✓ Recovery of glutamate transporter function

Figure 3. Mechanisms underlying depolarization and repolarization
It is already known that glutamate release plays a key role in generating the anoxic depolarization, although depolarization is related to many ions [4, 5]. (as shown in below) .

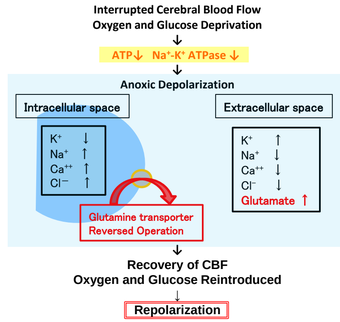
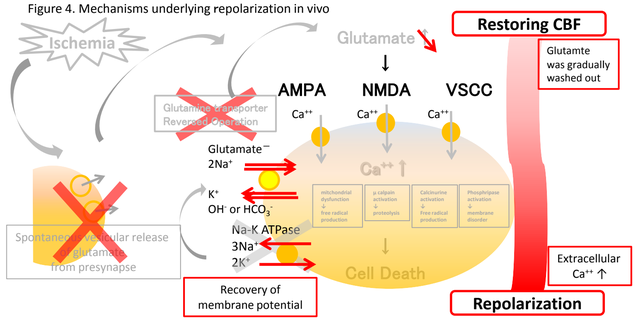


Figure 4. Mechanisms underlying repolarization in vivo



We could not pinpoint the amount of glutamate required to generate repolarization. Namely, the mechanisms of repolarization are due to nonselective recovery in the permeability of all participating ions and a decrease of glutamate[4, 5, 6] .

Conclusion

- **The extracellular glutamate level started to increase at the onset of ischemic depolarization.**
- **Hypothermia, which was induced upon the onset of ischemic depolarization, effectively suppressed the increase in extracellular glutamate levels.**
- **After repolarization, glutamate levels correlated with DC amplitudes.**

References

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Disclosures

Okayama University and Daiken Medical Co. (Osaka, Japan) hold patents related to the pharyngeal cooling. The laboratory is partially funded by Daiken Medical Co.