

**AU1 ▶** **Determination of the Target Temperature Required to Block Increases in Extracellular Glutamate Levels During Intraischemic Hypothermia**

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This study aimed to determine a target temperature for intraischemic hypothermia that can block increases in extracellular glutamate levels. Two groups of 10 rats each formed the normothermia and intraischemic hypothermia groups. Extracellular glutamate levels, the extracellular potential, and the cerebral blood flow were measured at the adjacent site in the right parietal cerebral cortex. Cerebral ischemia was induced by occlusion of the bilateral common carotid arteries and hypotension. In the intraischemic hypothermia group, brain hypothermia was initiated immediately after the onset of membrane potential loss. In the normothermia group, extracellular glutamate levels began to increase simultaneously with the onset of membrane potential loss and reached a maximum level of  $341.8 \pm 153.1 \mu\text{mol} \cdot \text{L}^{-1}$ . A decrease in extracellular glutamate levels was observed simultaneously with the onset of membrane potential recovery. In the intraischemic hypothermia group, extracellular glutamate levels initially began to increase, similarly to those in the normothermia group, but subsequently plateaued at  $140.5 \pm 105.4 \mu\text{mol} \cdot \text{L}^{-1}$ , when the brain temperature had decreased to  $<32.6^\circ\text{C} \pm 0.9^\circ\text{C}$ . A decrease in extracellular glutamate levels was observed simultaneously with the onset of membrane potential recovery, similarly to the findings in the normothermia group. The rate of decrease in extracellular glutamate levels was the same in both groups ( $-36.6$  and  $-36.0 \mu\text{mol} \cdot \text{L}^{-1}$  in the normothermia and intraischemic hypothermia groups, respectively). In conclusion, the target temperature for blocking glutamate release during intraischemic hypothermia was found to be  $32.6^\circ\text{C} \pm 0.9^\circ\text{C}$ . Our results suggest that the induction of intraischemic hypothermia can

**AU4 ▶** maintain low glutamate levels without disrupting glutamate reuptake. Institutional protocol number: OKU-2016146.

**AU5 ▶** **Keywords:** brain ischemia, glutamate, glutamate transporter, hypothermia, membrane potential, temperature

**Introduction**

**T**HE MECHANISMS OF NEUROLOGICAL DAMAGE after cardiac arrest involve two phases, namely primary and secondary injury. Primary injury is initiated by energy deprivation during the loss of membrane potential. It is well known that glutamate plays a key role in primary injury (Rothman and Olney, 1986; Choi and Rothman, 1990) because it exacerbates the intracellular calcium overload, which leads to secondary injury. Therefore, extracellular glutamate levels have a significant impact on the ultimate extent of cerebral damage. Secondary injury is gradually initiated by several cellular processes after the return of spontaneous circulation (ROSC), including oxidative stress, inflammation, and apoptosis (Doyle *et al.*, 2008; Brouns and De Deyn, 2009). The 2015 International Consensus on Cardiopulmonary

Resuscitation and Emergency Cardiovascular Care Science with Treatment Recommendations (Nolan *et al.*, 2015) recommends targeted temperature management between  $32^\circ\text{C}$  and  $36^\circ\text{C}$  for patients after ROSC. This management recommendation targets secondary damage; however, there is no consensus regarding target temperature for intraischemic hypothermia for primary injury.

Recently, it has become possible to reduce primary injury using intranasal cooling or pharyngeal cooling without disturbing the ROSC in clinical situations because brain temperature is selectively decreased (Castren *et al.*, 2010; Takeda *et al.*, 2012, 2014). Furthermore, it was reported that the early induction of hypothermia during cardiac arrest improves neurological outcomes in patients who undergo extracorporeal cardiopulmonary resuscitation (ECPR) (Nagao *et al.*, 2010). There is growing evidence that ECPR with hypothermia leads

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to a favorable neurological outcome regardless of patients being in the hospital or out of the hospital (Sakamoto *et al.*, 2014; Tan, 2017). All of these cooling methods can cool the brain during a cardiac arrest without disturbing the ROSC. Although it has been demonstrated that brain hypothermia induced before ischemia abolishes increases in extracellular glutamate levels (Busto *et al.*, 1989; Patel *et al.*, 1994), no studies have reported the target temperature for intraischemic hypothermia based on dynamic changes in glutamate levels.

The present study was designed to determine the target temperature for suppressing increases in extracellular glutamate levels during the loss and recovery of membrane potential in rat models. We serially measured glutamate levels every minute and continuously measured extracellular potential and cerebral blood flow (CBF) at the adjacent site in the right parietal cortex.

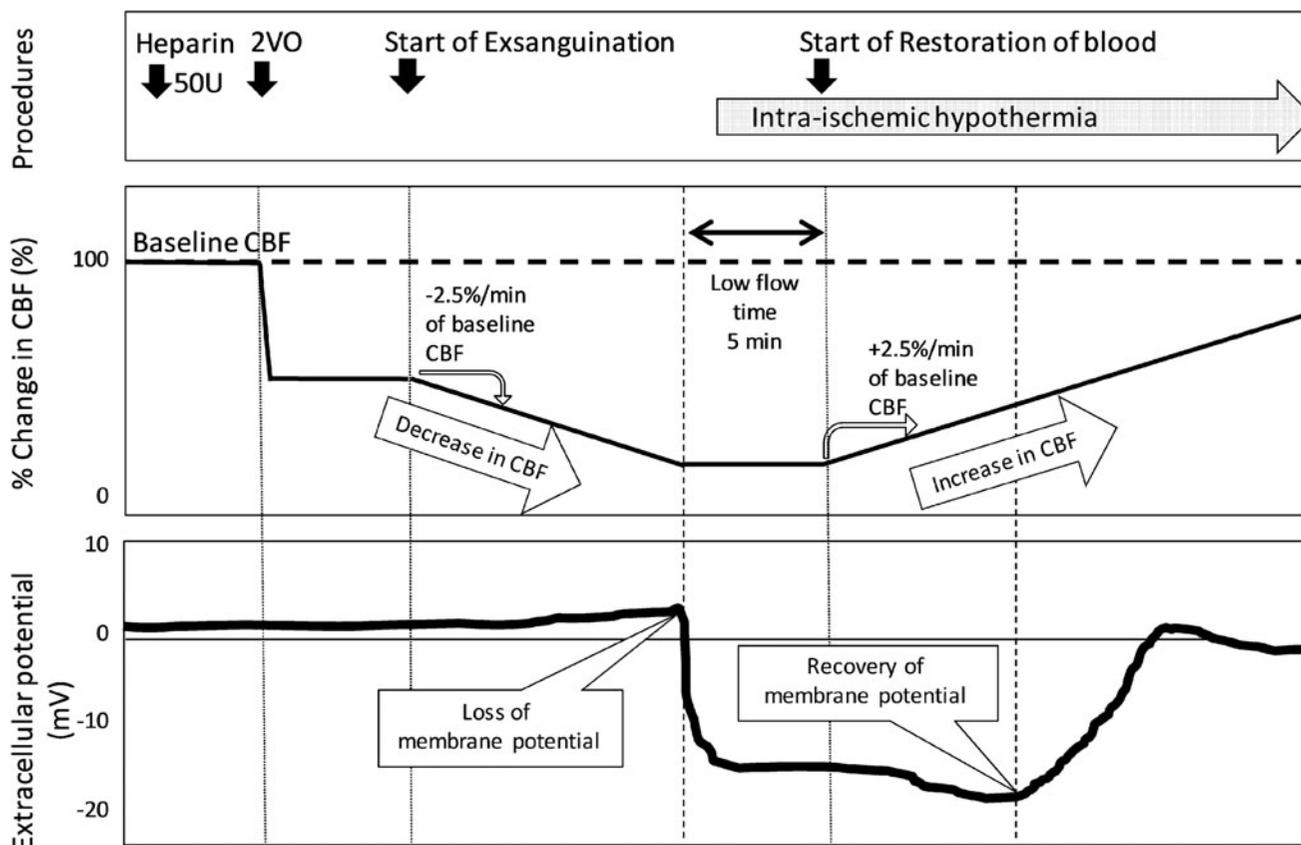
**Materials and Methods**

A total of 20 male Sprague-Dawley rats (Charles River Japan, Yokohama, Japan) weighing  $311 \pm 12$  g were used in this study. The sample size was estimated using a power analysis based on the results of a previous study using the same animal model (actual power, 0.85; total sample size, 9) (Mizoue *et al.*, 2015). The animals were housed under spe-

cific pathogen-free conditions in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health. Animals were fasted overnight before the experiments, although they had *ad libitum* access to water. All experiments were performed in accordance with the *National Institutes of Health Animal Care Guidelines* and were approved by the Animal Research Control Committee of Okayama University Medical School, Okayama City, Japan.

*General protocol*

The experiment was performed in a university hospital laboratory. The animals were divided into two groups of 10 rats each: a normothermia group and an intraischemic hypothermia group. Anesthesia was induced using 4% isoflurane in oxygen. After tracheal intubation and the initiation of artificial ventilation (SN-408-7; Shinano, Tokyo, Japan), anesthesia was maintained using 1.5% isoflurane in 60% oxygen balanced with nitrogen. Polyethylene catheters were placed in the right femoral artery for continuous monitoring of mean arterial blood pressure and blood sampling, and in the right femoral vein for administration of heparin and exsanguination. After placement of the animal in a stereotaxic apparatus (Narishige, Tokyo, Japan), a borosilicate glass electrode (tip diameter  $<5 \mu\text{m}$ ) was placed through a burr hole into the fifth layer of the right parietal



**FIG. 1.** Experimental protocol. The percent change in the CBF and extracellular potential are shown. The baseline CBF values were measured after surgery. The bilateral common carotid arteries were occluded 5 minutes after heparin injection (50 U). Five minutes later, CBF was continuously decreased by exsanguination of the venous blood at a rate of 2.5% of the baseline level until a sudden negative shift in the extracellular potential (loss of membrane potential) was observed. CBF was maintained at the level of membrane potential loss for 5 minutes, following which it was increased by the return of blood flow at the same rate. The shaded arrow region indicates the duration of brain hypothermia initiated immediately after membrane potential loss. CBF, cerebral blood flow.

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cerebral cortex (3 mm posterior and 3 mm right of the bregma, 0.75 mm below the cortical surface) for monitoring the extracellular potential. Adjacent to the extracellular electrode, a microdialysis probe (membrane length, 1.0 mm; molecular weight cutoff, 50,000; outer diameter, 220 μm [A-I-4-010; Eicom, Kyoto, Japan]) was placed 1.25 mm below the cortical surface for the serial measurement of glutamate levels, and a laser Doppler flow probe (FLO-C1; Omegawave, Tokyo, Japan) was placed on the dura for continuous monitoring of regional CBF. Brain temperature was monitored using a small thermocouple placed in the left parietal epidural space and controlled by a gentle flow of warmed saline coursing over the skull surface. Rectal temperature was monitored and maintained at 37.0°C ± 0.5°C using a heated water blanket.

vessel occlusion [2VO] ), CBF was decreased at a rate of 2.5% of the baseline level/min by draining the venous blood until a sudden negative shift in the extracellular potential (i.e., membrane potential loss) was observed. CBF was maintained at the level of membrane potential loss for 5 minutes, following which it was restored by the return of blood flow at the same rate. The beginning of membrane potential loss was defined as a sudden negative shift in the extracellular potential. The beginning of membrane potential recovery was defined as a positive shift in the extracellular potential for >1 minute.

Measurement of glutamate levels

The microdialysis probes were perfused with Ringer’s solution at a flow rate of 2 μL · min<sup>-1</sup> using an infusion pump (ESP-32; Eicom). The dialysate was automatically collected every minute using a fraction collector (EFC-82; Eicom) in a sample tube containing 10 μL of Ringer’s solution (total

Ischemia method

FI ► The experimental protocol is outlined in Figure 1. After occlusion of the bilateral common carotid arteries (two-

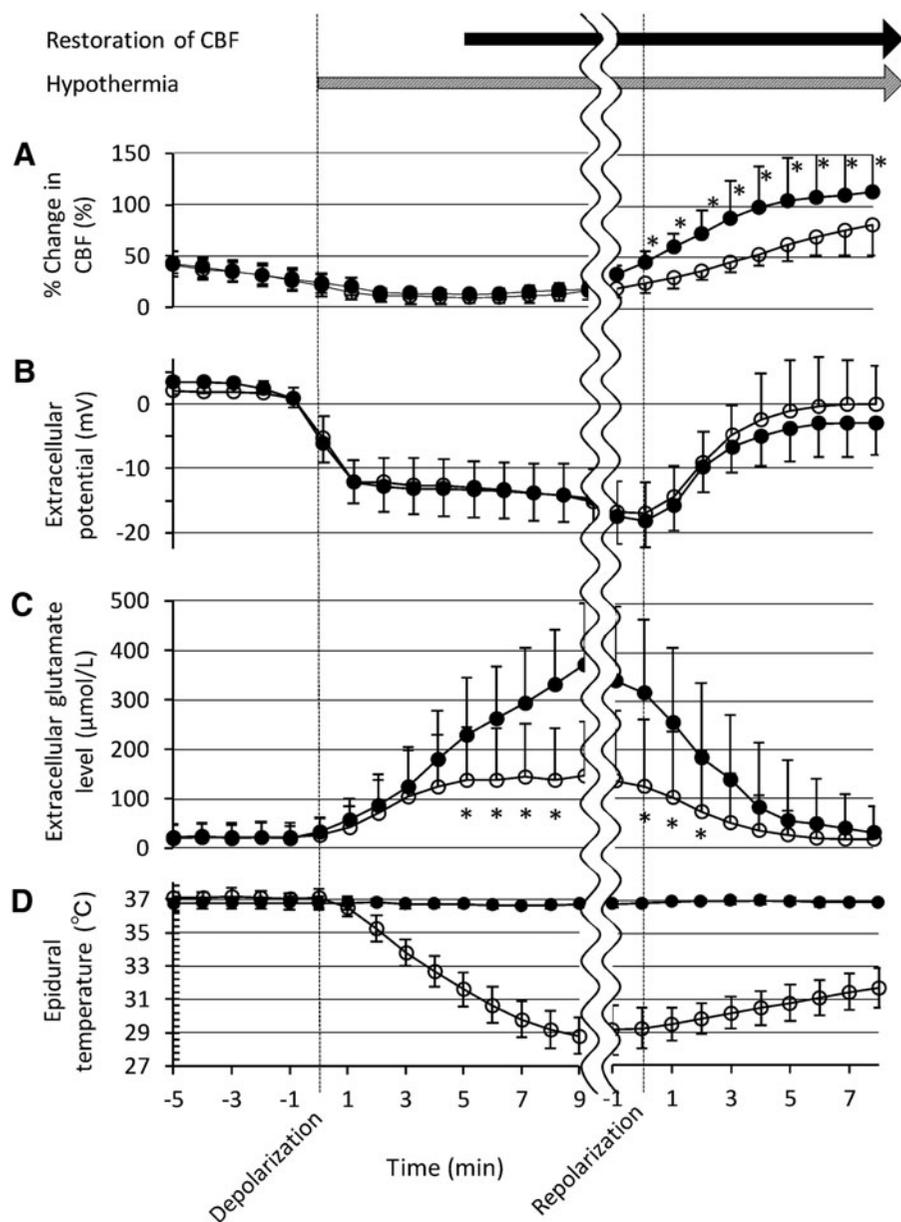


FIG. 2. Changes in the CBF (A), the extracellular potential (B), extracellular glutamate levels (C), and the epidural temperature (D). CBF is shown as a percentage change from the baseline CBF, and the extracellular potential is shown inversely from the bottom to the top. The X-axis represents time; the “depolarization” and “repolarization” points indicate the onset of membrane potential loss and membrane potential recovery, respectively. CBF, the extracellular potential, and the epidural temperature are shown as average values for 1 minute, corresponding to the period of collection of each glutamate sample. The filled circles and open circles indicate the values for the normothermia and intraischemic hypothermia groups, respectively. \*p < 0.05 compared with the normothermia group. CBF, cerebral blood flow.

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12  $\mu\text{L}$  for each sample) to prevent evaporation. Glutamate was quantified using an electrochemical detector (ECD) with high-performance liquid chromatography and a computerized control system (Nanospace Syscon 21; Shiseido, Tokyo, Japan). Glutamate was isolated from the samples using a separation column (4.6  $\times$  150 mm, 4  $\mu\text{m}$ , GU-GEL; Eicom) and oxidized by an immobilized enzyme column (3.0  $\times$  4.0 mm, 37–74  $\mu\text{m}$ , E-ENZYMOPAC; Eicom) to hydrogen peroxide, which was detected by the ECD. The mobile phase consisted of 250  $\text{mg}\cdot\text{L}^{-1}$  cetrimonium bromide ( $\text{C}_{19}\text{H}_{42}\text{BrN}$ ) and 0.05  $\text{mg}\cdot\text{L}^{-1}$  EDTA sodium in 60  $\text{mmol}\cdot\text{L}^{-1}$   $\text{NH}_4\text{Cl}\text{-NH}_4\text{OH}$  solution.

**Cooling method**

Nasopharyngeal cooling (Hagioka *et al.*, 2003) was initiated immediately after the onset of membrane potential loss. Cannulae (20-gauge) were inserted into both nasal cavities to a depth of 5 mm. Cold physiological saline (5°C) was infused at a rate of 100  $\text{mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  of body weight using a roller pump until the epidural temperature had decreased to 31°C. The cold saline flowed out of the oral cavity and was aspirated by a suction device to prevent the animal from becoming wet. This method enabled a rapid decrease in the temperature of the entire brain without a decrease in the body temperature. Hypothermia was maintained until the end of membrane potential recovery. The rectal temperature was maintained at 37.0°C  $\pm$  0.5°C during this period.

**Statistical analyses**

All values are expressed as means  $\pm$  standard deviations. The glutamate and extracellular potential data were analyzed using repeated-measures analysis of variance followed by Fisher’s *post hoc* test. All other statistical analyses were performed using Student’s *t*-test (two tailed). A *p*-value of <0.05 was considered statistically significant in all tests.

**Results**

All parameters were maintained within normal ranges before the initiation of ischemia. Changes in CBF, the extracellular potential, extracellular glutamate levels, and the epidural temperature are shown in Figure 2.

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In the normothermia group, the CBF threshold for membrane potential loss was 21.2%  $\pm$  7.9% of the control level (Fig. 2A). Extracellular glutamate levels (preischemic value, 32.1  $\pm$  34.4  $\mu\text{mol}\cdot\text{L}^{-1}$ ) began to increase simultaneously with the onset of membrane potential loss and reached a maximum level of 341.8  $\pm$  153.1  $\mu\text{mol}\cdot\text{L}^{-1}$  (Fig. 2B, C). A decrease in extracellular glutamate levels was observed simultaneously with the onset of membrane potential recovery. The CBF threshold for membrane potential recovery was 44.9%  $\pm$  21.6% of the control level (Fig. 2A).

In the intraischemic hypothermia group, the CBF threshold for membrane potential loss was 20.8%  $\pm$  10.5% of the control level (*p* = 0.94 vs. normothermia group; Fig. 2A). Extracellular glutamate levels (preischemic value, 28.1  $\pm$  39.8  $\mu\text{mol}\cdot\text{L}^{-1}$ ; *p* = 0.75) initially began to increase, similarly to those in the normothermia group, and subsequently plateaued at 140.5  $\pm$  105.4  $\mu\text{mol}\cdot\text{L}^{-1}$ , when the brain temperature had decreased to <32.6°C  $\pm$  0.9°C (Fig. 2B–D). The 95% confidence interval of the target temperature for intraischemic hypothermia required

to block the increase in extracellular glutamate levels in rats was 32.0–33.2°C. A decrease in extracellular glutamate levels was observed simultaneously with the onset of membrane potential recovery, similarly to the findings in the normothermia group. The CBF threshold for membrane potential recovery (26.3%  $\pm$  9.3% of the control level) was significantly lower in this group than in the normothermia group (*p* = 0.02; Fig. 2A). Extracellular glutamate levels at this point were also significantly lower (125  $\pm$  96  $\mu\text{mol}\cdot\text{L}^{-1}$ ) than those in the normothermia group (317  $\pm$  155  $\mu\text{mol}\cdot\text{L}^{-1}$ ; *p* = 0.004; Fig. 2C).

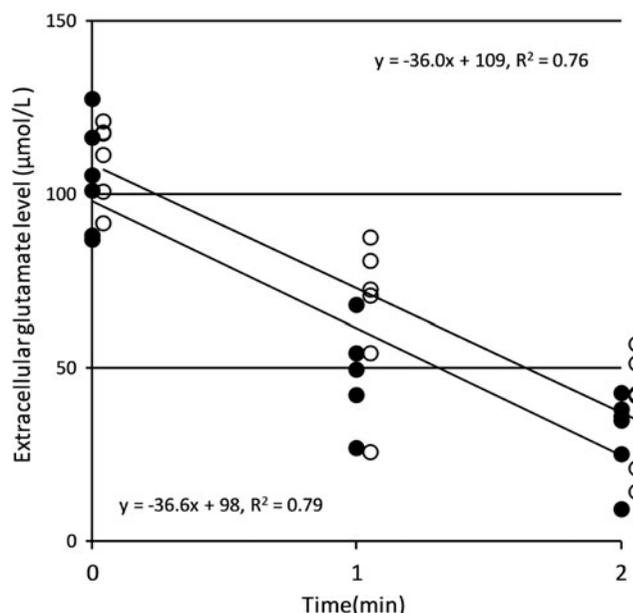
Extracellular glutamate levels decreased from 104  $\pm$  16 to 31  $\pm$  12  $\mu\text{mol}\cdot\text{L}^{-1}$  in the normothermia group and from 112  $\pm$  12 to 40  $\pm$  17  $\mu\text{mol}\cdot\text{L}^{-1}$  in the intraischemic hypothermia group, with decrease rates of –36.6 and –36.0  $\mu\text{mol}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$ , respectively (Fig. 3).

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**Discussion**

To the best of our knowledge, the present study is the first to determine the target temperature for intraischemic hypothermia in a rat model. By decreasing the brain temperature to <32.6°C  $\pm$  0.9°C (95% confidence interval: 32.0–33.2°C), intraischemic hypothermia can block increases in extracellular glutamate levels. Table 1 summarizes the results of previous studies investigating the effects of hypothermia initiated “before ischemia” on glutamate levels. Extracellular glutamate levels did not increase when the brain temperature was <33°C, regardless of the animal species (e.g., rats and rabbits), region of the brain (e.g., striatum, dorsal hippocampus, and left parietal cortex), method of inducing brain ischemia (e.g., four-VO plus hypotension, neck tourniquet plus hypotension, and cardiac arrest), or the duration of ischemia (e.g., 5, 7.5, 15, and

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**FIG. 3.** Rate of decrease in extracellular glutamate levels. The filled circles and open circles indicate the values for the normothermia and intraischemic hypothermia groups, respectively. The rates of decrease in extracellular glutamate levels, shown by the inclination of each line, are –36.6 and –36.0  $\mu\text{mol}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$  in the normothermia and intraischemic hypothermia groups, respectively.

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TABLE 1. EFFECT OF HYPOTHERMIA ON EXTRACELLULAR GLUTAMATE CONCENTRATION

First author	Model	Brain region	Duration of ischemia (min)	Increase in extracellular glutamate level
Globus	Rat, 4VO+hypotension	Striatum	20	710% (36), 150% (33), 120% (30)
Busto	Rat, 4VO+hypotension	Striatum	20	710% (36), 160% (33), 130% (30)
Illievich	Rabbit, neck tourniquet+hypotension	Dorsal hippocampus	15	1100% (38), 110% (32), 100% (28)
Illievich	Rabbit, neck tourniquet+hypotension	Dorsal hippocampus	7.5	580% (37), 150% (32)
Nakashima	Rat, cardiac arrest	Left parietal cortex	Not resuscitated	550% (38), 110% (28)
Takata	Rat, cardiac arrest	Left parietal cortex	5	230% (37), 100% (31)

Glutamate level is expressed relative to the preischemic value, shown as 100%. Values in parentheses indicate temperature in degrees centigrade. The increase in the extracellular glutamate level is greatly reduced when the brain temperature is less than 33°C. 4VO, four-vessel occlusion.

20 minutes) (Globus *et al.*, 1988; Busto *et al.*, 1989; Illievich *et al.*, 1994a, 1994b; Nakashima and Todd, 1996; Takata *et al.*, 2005). These observations suggest that the mechanisms of glutamate release are common and blocked when the brain temperature is <33°C, regardless of whether hypothermia is initiated before or after the onset of ischemia.

In the present study, intraischemic hypothermia did not decrease extracellular glutamate levels. A decrease in extracellular glutamate levels was observed simultaneously with the onset of membrane potential recovery. Because the increase in extracellular glutamate levels during ischemia is caused by the reverse operation of glutamate transporters (Rossi *et al.*, 2000; Mitani and Tanaka, 2003), it has been hypothesized that intraischemic hypothermia can block this reverse operation. On the contrary, decreases in extracellular glutamate levels are caused by the forward operation of glutamate transporters that use the Na<sup>+</sup> electrochemical gradient as a driving force, which is generated by Na<sup>+</sup>/K<sup>+</sup>-ATPase (Barbour *et al.*, 1988; Nicholls and Attwell, 1990; O’Kane *et al.*, 1999). Accordingly, the decrease in extracellular glutamate levels is an energy-dependent process, and the recovery of membrane potential is essential for this decrease.

In the present study, the rates of decrease in extracellular glutamate levels were the same in the normothermia (−36.6 μmol·L<sup>−1</sup>·min<sup>−1</sup>) and in intraischemic hypothermia (−36.0 μmol·L<sup>−1</sup>·min<sup>−1</sup>) groups. These observations suggest that although in intraischemic hypothermia blocked an increase in extracellular glutamate levels, it did not disrupt their decrease. Increases and decreases in extracellular glutamate levels primarily depend on the reverse and forward operations of glutamate transporters, respectively, under ischemic conditions (Rossi *et al.*, 2000; Mitani and Tanaka, 2003). Because it has been reported that reverse operation does not reflect the process of forward operation (i.e., differences in the degree of Na<sup>+</sup> voltage dependency in the site of the K<sup>+</sup> relocation branch) (Zhang *et al.*, 2007; Grewer *et al.*, 2008), the sensitivity of each process to hypothermia appears to be different. Thus, in intraischemic hypothermia can be safely implemented without disrupting decreases in extracellular glutamate levels.

The CBF thresholds for membrane potential recovery were significantly lower in the intraischemic hypothermia group than in the normothermia group (p=0.02). Extracellular glutamate levels at this point were also significantly lower in

the in intraischemic hypothermia group than in the normothermia group (p=0.004). Because the glutamate transporter forms a macromolecule with Na<sup>+</sup>/K<sup>+</sup>-ATPase and is functionally linked to it (Rose *et al.*, 2009), the energy necessary for membrane potential recovery is lower under low extracellular glutamate levels. In clinical situations, the early initiation of intraischemic hypothermia blocks an increase in extracellular glutamate levels and thus facilitates membrane potential recovery.

This study has several limitations. First, CBF was decreased by a rate of 2.5% of the baseline per minute by draining blood. This gradual decrease in CBF did not mimic the actual changes occurring in CBF during cardiac arrest. Although cerebral ischemia suddenly occurs after cardiac arrest, the metabolic changes in neuronal cells gradually progress for ~2 minutes until the onset of membrane potential loss. A gradual change in CBF was necessary for accurate determination of the target temperature threshold for suppressing the increase in extracellular glutamate levels and for determination of the CBF threshold for membrane potential recovery.

Second, in the present study, we performed selective brain cooling using nasopharyngeal cooling, and the brain temperature was measured in the epidural space. Since it has been reported that the epidural temperature is lower than the subcortical temperature by 1.0°C during the nasopharyngeal cooling of rats (Hagioka *et al.*, 2003) and primates (Takeda *et al.*, 2012), we may need to decrease the epidural temperature by a further 1.0°C to protect subcortical regions. On the contrary, in whole body cooling, it seems that differences in temperature between cortical and subcortical regions are relatively small. However, since it has been reported that subcortical temperature is higher than the rectal or esophageal temperature by 0.34°C (Coppler *et al.*, 2016), body temperature may also need to be controlled to compensate for this difference.

Third, in the present study, the target temperature for suppressing increases in extracellular glutamate levels was observed. However, this could be different from the target temperature for suppressing neuronal damage, since there is a clear difference in the mechanisms of neuronal damage between the ischemic period and the recirculation period (Dirnagl *et al.*, 1999). For example, the formation of oxygen free radicals is observed only during the recirculation period and is

reported to be suppressed by lowering brain temperature to less than 30°C (Globus *et al.*, 1995). In contrast, as shown in the present study, glutamate is released only during the ischemic period. The target temperature for suppressing neuronal damage would be different between the ischemic period and the recirculation period.

**Conclusions**

The target temperature for blocking glutamate release during intraischemic hypothermia was found to be 32.6°C ± 0.9°C (95% confidence interval: 32.0–33.2°C). Our results suggest that earlier induction of intraischemic hypothermia can maintain low glutamate levels without disrupting glutamate reuptake. Membrane potential recovery is essential for a decrease in extracellular glutamate levels. Because earlier initiation of intraischemic hypothermia can suppress extracellular glutamate levels, the CBF threshold for membrane potential recovery can also be lowered.

**Acknowledgments**

This study was supported by a grant from the Ministry of Health, Labour and Welfare of Japan (26293345) and a grant from Daiken Medical Co. No funding sources were involved in the study design; collection, analysis, and interpretation of the data; writing of the article; or in the decision to submit the article for publication.

**Author Disclosure Statement**

Dr. Takeda is developing a pharyngeal cooling system to decrease the brain temperature during resuscitation, particularly before the return of spontaneous circulation. His institution received grant support and royalties from Daiken Medical. No competing financial interests exist for the remaining authors.

**References**

Barbour B, Brew H, Attwell D. Electrogenic glutamate uptake in glial cells is activated by intracellular potassium. *Nature* 1988;335:433–435.

Brouns R, De Deyn PP. The complexity of neurobiological processes in acute ischemic stroke. *Clin Neurol Neurosurg* 2009;111:483–495.

Busto R, Globus MY, Dietrich WD, Martinez E, Valdes I, Ginsberg MD. Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. *Stroke* 1989;20:904–910.

Castren M, Nordberg P, Svensson L, Taccone F, Vincent JL, Desruelles D, Eichwede F, Mols P, Schwab T, Vergnion M, Storm C, Pesenti A, Pacht J, Guerisse F, Elste T, Roessler M, Fritz H, Durnez P, Busch HJ, Inderbitzen B, Barbut D. Intra-arrest transnasal evaporative cooling: a randomized, pre-hospital, multicenter study (PRINCE: Pre-ROSC IntraNasal Cooling Effectiveness). *Circulation* 2010;122:729–736.

Choi DW, Rothman SM. The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. *Annu Rev Neurosci* 1990;13:171–182.

Coppler PJ, Marill KA, Okonkwo DO, Shutter LA, Dezfulian C, Rittenberger JC, Callaway CW, Elmer J. Concordance of brain and core temperature in comatose patients after cardiac arrest. *Ther Hypothermia Temp Manag* 2016;6:194–197.

Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischemic stroke: an integrated view. *Trends Neurosci* 1999;22:391–397.

Doyle KP, Simon RP, Stenzel-Poore MP. Mechanisms of ischemic brain damage. *Neuropharmacology* 2008;55:310–318.

Globus MY, Busto R, Dietrich WD, Martinez E, Valdes I, Ginsberg MD. Intra-ischemic extracellular release of dopamine and glutamate is associated with striatal vulnerability to ischemia. *Neurosci Lett* 1988;91:36–40.

Globus MY, Busto R, Lin B, Schnippering H, Ginsberg MD. Detection of free radical activity during transient global ischemia and recirculation: effects of intraischemic brain temperature modulation. *J Neurochem* 1995;65:1250–1256.

Grewer C, Gameiro A, Zhang Z, Tao Z, Braams S, Rauen T. Glutamate forward and reverse transport: from molecular mechanism to transport-mediated release after ischemia. *IUBMB Life* 2008;60:609–619.

Hagioka S, Takeda Y, Takata K, Morita K. Nasopharyngeal cooling selectively and rapidly decrease brain temperature and attenuate neuronal damage, even if initiated at the onset of cardiopulmonary resuscitation in rats. *Crit Care Med* 2003;31:2502–2508.

Illievich UM, Zornow MH, Choi KT, Scheller MS, Strnat MAP. Effects of hypothermic metabolic suppression on hippocampal glutamate concentrations after transient global cerebral ischemia. *Anesth Analg* 1994a;78:905–911.

Illievich UM, Zornow MH, Choi KT, Strnat MAP, Scheller MS. Effects of hypothermia or anesthetics on hippocampal glutamate and glycine concentrations after repeated transient global cerebral ischemia. *Anesthesiology* 1994b;80:177–186.

Mitani A, Tanaka K. Functional changes of glutamate transporter GLT-1 during ischemia: an in vivo study in the hippocampal CA1 of normal mice and mutant lacking GLT-1. *J Neurosci* 2003;23:7176–7182.

Mizoue R, Takeda Y, Sato S, Takata K, Morimatsu H. Cerebral blood flow threshold is higher for membrane repolarization than for depolarization and is lowered by intraischemic hypothermia in rats. *Crit Care Med* 2015;43:e350–e355.

Nagao K, Kikushima K, Watanabe K, Tachibana E, Tominaga Y, Tada K, Ishii M, Chiba N, Kasai A, Soga T, Matsuzaki M, Nishikawa K, Tateda Y, Ikeda H, Yagi T. Early induction of hypothermia during cardiac arrest improves neurological outcomes in patients with out-of-hospital cardiac arrest who undergo emergency cardiopulmonary bypass and percutaneous coronary intervention. *Circ J* 2010;74:77–85.

Nakashima K, Todd MM. Effects of hypothermia, pentobarbital and isoflurane on postdepolarization amino acid release during complete cerebral ischemia. *Anesthesiology* 1996;85:161–168.

Nicholls D, Attwell D. The release and uptake of excitatory amino acids. *Trends Pharmacol Sci* 1990;11:462–468.

Nolan JP, Hazinski MF, Aickin R, Bhanji F, Billi JE, Callaway CW, Castren M, de Caen AR, Ferrer JM, Finn JC, Gent LM, Griffin RE, Iverson S, Lang E, Lim SH, Maconochie IK, Montgomery WH, Morley PT, Nadkarni VM, Neumar RW, Nikolaou NI, Perkins GD, Perlman JM, Singletary EM, Soar J, Travers AH, Welsford M, Wyllie J, Zideman DA. Part 1: executive summary: 2015 International consensus on cardiopulmonary resuscitation and emergency cardiovascular care science with treatment recommendations. *Resuscitation* 2015;95:e1–e31.

O’Kane RL, Martinez-Lopez I, DeJoseph MR, Vina JR, Hawkins RA. Na<sup>+</sup>-dependent glutamate transporters (EAAT1, EAAT2, and EAAT3) of the blood-brain barrier. *J Biol Chem* 1999;274:31891–31895.

THE TARGET TEMPERATURE TO BLOCK GLUTAMATE RELEASE

Patel PM, Drummond JC, Cole DJ, Yaksh TL. Differential temperature sensitivity of ischemia-induced glutamate release and eicosanoid production in rats. *Brain Res* 1994;650:205–211.

Rose EM, Koo JC, Antflick JE, Ahmed SM, Angers S, Hampson DR. Glutamate transporter coupling to Na, K-ATPase. *J Neurosci* 2009;29:8143–8155.

Rossi DJ, Oshima T, Attwell D. Glutamate release in severe brain ischemia is mainly by reversed uptake. *Nature* 2000;403:316–321.

Rothman SM, Olney JW. Glutamate and the pathophysiology of hypoxic-ischemic brain damage. *Ann Neurol* 1986;19:105–111.

Sakamoto T, Morimura N, Nagao K, Asai Y, Yokota H, Nara S, Hase M, Tahara Y, Atsumi T. Extracorporeal cardiopulmonary resuscitation versus conventional cardiopulmonary resuscitation in adults with out-of-hospital cardiac arrest: a prospective observational study. *Resuscitation* 2014;85:762–768.

Takata K, Takeda Y, Sato T, Nakatsuka H, Yokoyama M, Morita K. Effects of hypothermia for a short period on histologic outcome and extracellular glutamate concentration during and after cardiac arrest in rats. *Crit Care Med* 2005;33:1340–1345.

Takeda Y, Hashimoto H, Fumoto K, Danura T, Naito H, Morimoto N, Katayama H, Fushimi S, Matsukawa A, Ohtsuka A, Morita K. Effects of pharyngeal cooling on brain temperature in primates and humans. *Anesthesiology* 2012;117:117–125.

Takeda Y, Kawashima T, Kiyota K, Oda S, Morimoto N, Kobata H, Isobe H, Honda M, Fujimi S, Onda J, I S, Sakamoto T, Ishikawa M, Nakano H, Sadamitsu D, Kishikawa M, Kinoshita K, Yokoyama T, Harada M, Kitaura M, Ichihara K, Hashimoto H, Tsuji H, Yorifuji T, Nagano O, Katayama H, Ujike Y, Morita K. Feasibility study of immediate pharyngeal cooling initiation in cardiac arrest patients after arrival at the emergency room. *Resuscitation* 2014;85:1647–1653.

Tan BK. Extracorporeal membrane oxygenation in cardiac arrest. *Singapore Med J* 2017;58:446–448.

Zhang Z1, Tao Z, Gameiro A, Barcelona S, Braams S, Rauen T, Grever C. Transport direction determines the kinetics of substrate transport by the glutamate transport EAAC1. *Proc Natl Acad Sci USA* 2007;104:18025–18030.

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