Title: Cerebral blood flow threshold is higher for membrane repolarization than for depolarization and is lowered by intraischemic hypothermia in rats

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Key words: cerebral ischemia, cardiopulmonary resuscitation, repolarization, hypothermia, cerebral blood flow, threshold

Abstract

Objective: To evaluate the cerebral blood flow (CBF) threshold for membrane depolarization and repolarization and the effects of brain hypothermia at this threshold.

Design: Prospective animal study.

Setting: Experimental laboratory in a university hospital.

Subjects: Male Sprague-Dawley rats (n = 40).

Intervention: CBF and membrane depolarization and repolarization in the cerebral cortex were simultaneously monitored by laser Doppler and extracellular potential, respectively. Following bilateral occlusion of the common carotid arteries, CBF was decreased by draining blood at a rate of 2.5% of the control level/min until membrane depolarization was initiated. At 5 and 10 min (Normothermia5 and Normothermia10 groups, respectively) after depolarization onset, CBF was restored at the same rate until membrane repolarization was observed. In some animals, intra-ischemic brain hypothermia targeting 31°C was initiated immediately after the onset of depolarization (Hypothermia5 and Hypothermia10 groups).

Measurements and main results: The CBF threshold for repolarization (46.5% ± 12%) was significantly higher than for depolarization (18.9% ± 4.8%; $P < 0.01$) in the Normothermia5 group, and was further increased to 61.5% ± 14% ($P < 0.01$) in the Normothermia10 group. With initiation of hypothermia, the CBF threshold for membrane repolarization was suppressed to 33.8% ± 10% in the Hypothermia5 group ($P < 0.01$ vs. Normothermia5 group) and was

unaltered by prolongation of ischemia (Hypothermia10 group, 36.6% ± 6%).

Conclusions: CBF thresholds were significantly higher for repolarization than for depolarization, and were further increased by prolonged ischemia. Intra-ischemic brain hypothermia decreased the repolarization threshold and abrogated the increase in the repolarization threshold caused by prolonged ischemia.

INTRODUCTION

Cardiopulmonary resuscitation (CPR) provides cerebral blood flow (CBF) following cardiac arrest. Several studies have reported that the CBF supplied by manual chest compressions is 20%–40% that of the normal level (1–3). This flow seems higher than the CBF threshold for ischemic depolarization, which has been reported in several animal studies (4–7). Therefore, if adequate CPR is performed immediately after cardiac arrest, it would be possible to prevent ischemic depolarization.

Brain ischemia leads to reduced ATP production and sodium-potassium pump failure, which subsequently induces ischemic depolarization (8), the duration of which is an important determinant of the severity of neuronal injury at early stages (9, 10). A better neurological outcome can be expected by averting ischemic depolarization via CPR; however, this is often initiated belatedly, since depolarization occurs within minutes of ischemia (11, 12). Even when this is not the case, inadequate CPR or the interruption or decrease in quality of chest compressions (for instance, due to rescuer fatigue) (13) can lead to decreased CBF and consequent ischemic depolarization.

Should ischemic depolarization occur during CPR, it is imperative to restore the membrane potential as quickly as possible. However, since the CBF threshold for membrane repolarization has not been studied, it is not known whether the membrane potential is restored in neurons during chest compressions or after the return of spontaneous circulation (ROSC).

Therapeutic hypothermia is an effective method for minimizing post-resuscitation injury after cardiac arrest (14); findings from clinical and animal studies suggest that intra-ischemic hypothermia improves neurological outcome (15–17). However, the neuroprotective effects are greatly reduced if the onset of hypothermia after ischemia is delayed (18, 19), although the underlying mechanisms have not yet been elucidated. Since hypothermia helps reduce cerebral metabolic rate (20, 21), intra-ischemic hypothermia may act by decreasing the CBF threshold for membrane repolarization.

This study was designed to elucidate the CBF thresholds for repolarization. Effect of intra-ischemic brain hypothermia on the threshold was also examined. Membrane depolarization and repolarization were assessed by measuring the extracellular potential, which can detect the changes in extracellular potential associated with ischemic depolarization (22).

MATERIAL AND METHODS

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.

General procedure

Male Sprague-Dawley rats (n = 40) (Charles River Japan, Yokohama, Japan) weighing 293 ± 14 g were used in this study. Animals fasted overnight before the experiment but had free access to water. Anesthesia was induced with 4% isoflurane in oxygen; after tracheal intubation and initiation of artificial ventilation (SN-480-7; Shinano, Tokyo, Japan), anesthesia was maintained with 1.5% isoflurane in 60% oxygen. During the experiment, body temperature was monitored with a rectal probe and maintained at 37.0 ± 0.5°C using a heated water blanket. A PE50 polyethylene catheter was placed in the right femoral artery and vein to continuously monitor arterial blood pressure and permit blood withdrawal during controlled hemorrhagic hypotension, respectively. A loose ligature was placed around each common carotid artery. After placement of the animal in a stereotaxic instrument (Narishige, Tokyo, Japan), burr holes (1-mm diameter) were drilled into both parietal bones. A borosilicate glass electrode (tip diameter < 5 μm) was placed in the fifth layer of the cerebral cortex (3 mm to the left of the sagittal line, 3 mm posterior to bregma, and 750 μm below the cortical surface) through a dural incision to measure the extracellular potential. A laser-Doppler flow (LDF) probe (OmegaFlo

FLO-C1, Omegawave, Tokyo, Japan) was placed on the surface of the thinly shaved left temporal bone adjacent to the glass electrode to continuously monitor regional CBF. Brain temperature was measured using a small thermocouple (500-μm diameter) placed in the right epidural space. After measuring physiological variables, 50 U of heparin were injected intravenously to prevent clotting of the drainage blood.

CBF thresholds for membrane depolarization and repolarization

Baseline CBF values were measured after the surgery. Starting from 5 min after occlusion of both common carotid arteries (2VO), CBF was decreased continuously at a speed of 2.5% of the baseline level/min by draining the venous blood through the right femoral vein catheter until a sudden negative shift in extracellular potential was observed. CBF was maintained at this level of depolarization for 5 or 10 min, and then restored at the same rate by returning blood until a positive shift in extracellular potential occurred. Animals were sacrificed when membrane potential was recovered to preischemic level.

The CBF threshold was defined as a sudden negative shift in extracellular potential for depolarization and the start of a continuous increase (>1 min) of extracellular potential for repolarization, because in pilot studies, after increasing for >1 min the extracellular potential continued to increase until the preischemic level was attained.

Experimental groups

Animals were divided into four groups (10 animals in each group) according to the epidural

temperature and low flow time (defined as the period from membrane depolarization to the return of venous blood). The experimental groups and procedures are outlined in Figure 1. The Normothemia5 and Normothermia10 groups had normal brain temperature with low flow times of 5 and 10 min, respectively; in Hypothermia5 and Hypothermia10 groups, brain hypothermia was initiated immediately after depolarization and low flow times were 5 and 10 min, respectively.

Nasopharyngeal cooling

In the Hypothermia5 and Hypothemia10 groups, selective brain hypothermia was initiated by nasopharyngeal cooling (23) immediately after the onset of depolarization. This method is designed to allow heat exchange with the bilateral carotid arteries at the pharynx. Briefly, 20-gauge cannulas were inserted into both nasal cavities to a depth of 5 mm. Cold physiologic saline (5°C) was infused at a rate of 100 $ml \cdot min^{-1} \cdot kg^{-1}$ body weight using a roller pump until the epidural temperature decreased to 31°C. The cold saline flowed out of the oral cavity and was aspirated by a suction device so that the animal did not get wet. This method enabled the temperature of the whole brain to be rapidly decreased without also decreasing the body temperature. Hypothermia was maintained until the beginning of repolarization. The rectal temperature was maintained at 37.0°C ± 0.5°C during this period.

Statistical analysis

Values are expressed as the mean ± standard deviation (SD). Blood gases and pH were

measured using the α-stat method. All statistical comparisons were performed with the use of Student's t test or analysis of variance followed by the Tukey–Kramer test for multiple comparisons. $P < 0.05$ was considered significant in all tests.

RESULTS

All physiological measurements obtained before ischemia were within normal ranges. Figure 2 shows representative changes in CBF and extracellular potential. Arrows show the time of depolarization and start of repolarization. Starting from the onset of repolarization, the extracellular potential increased continuously and returned to the preischemic level.

CBF thresholds for membrane depolarization are shown in Figure 3. The values were almost same and no significant differences were observed between groups ($P = 0.75$). The mean CBF threshold for depolarization was 18.9% ± 4.8% of the baseline value.

The CBF thresholds for membrane repolarization are shown in Figure 4. The CBF thresholds for repolarization were significantly higher than the thresholds for depolarization in all groups ($P < 0.01$). The CBF thresholds for repolarization in normothermia (Normothermia5 and Normothermia10) groups were 46.5% ± 11.8% and 61.5% ± 13.6% of the control level after 5 and 10 min of low flow time, respectively. Prolonging the low flow time significantly increased the threshold ($P < 0.01$, Normothemia5 vs Normothermia10).

In the hypothermia groups, epidural temperature declined sharply with the initiation of nasopharyngeal cooling, reaching 31°C at 4.9 ± 1.0 min without a corresponding change in rectal temperature. The CBF thresholds for repolarization in hypothermia (Hypothermia5 and Hypothermia10) groups were 33.8 ± 10% ($P < 0.01$, compared to Normothermia5) and 36.6% ± 6% ($P < 0.01$, compared to Normothermia10) of control level after 5 and 10 min of low flow

time, respectively. Prolonging the low flow time did not increase CBF threshold for repolarization in the hypothermia groups ($P > 0.05$).

DISCUSSION

This is the first study to measure the CBF threshold for membrane repolarization following ischemia, which were 46.5% ± 12% and 61.5% ± 13.6% of the control level after 5 and 10 min of low flow time, respectively, and were significantly higher than corresponding thresholds for depolarization (18.9% ± 4.8%). These results indicate that more CBF is required to restore the membrane potential than to maintain it.

Several studies have reported the CBF threshold for depolarization in rats. Harris et al. (5) reported that the threshold was 19.0% that of the control level in the rat bilateral common carotid artery occlusion plus hypotension model using the hydrogen clearance method, while Takeda et al. (7) reported a threshold of 23.9% that of the control level in the rat middle cerebral artery occlusion model using the autoradiographic method. Thus, our results are consistent with the results of previous studies.

Most studies that have measured the CBF supplied by chest compressions showed that it was almost 20%–40% of the normal level if vasopressors or other devices were not used (1–3), which is higher than the CBF threshold for ischemic depolarization measured in the present study. Therefore, if adequate CPR is performed before the onset of depolarization, the CBF supplied by chest compressions will likely prevent ischemic depolarization (Figure 5A, a).

However, once ischemic depolarization has occurred, it would be difficult to restore the membrane potential by manual chest compressions alone since the CBF threshold for

repolarization seems to be higher than the CBF supplied by manual chest compressions (Figure 5A, b). In addition, prolonged depolarization increases the repolarization threshold and makes it more difficult to restore the membrane potential. Delayed onset, interruption of CPR, or even rescuer fatigue would lead to depolarization of neuronal cells, and consequently we may not able to repolarize neuronal cells until achieving ROSC. This may demonstrate the mechanisms by which some patients do not show good neurological outcomes in spite of receiving bystander CPR. Our results indicate that it is obviously important to prevent the occurrence of ischemic depolarization. Therefore, early initiation, high quality, and minimum interruption of CPR are essential for good neurological recovery.

In the present study, intra-ischemic hypothermia induced immediately after depolarization decreased CBF thresholds for repolarization. These results indicate that hypothermia during ischemic depolarization may enable neuronal cells to repolarize with manual chest compressions. Moreover since it has been reported that hypothermia delays the onset of neuronal damage during ischemic depolarization, initiation of hypothermia during ischemic depolarization may increase the therapeutic time window for membrane repolarization.

Although there are no data from humans, onset time of depolarization after ischemia has been reported to be 100 s in rats (10, 11). In this context, a prospective study which observed the outcome among 34,605 out-of-hospital cardiac arrest patients reported that patients who receive bystander CPR within 5 min of ischemia showed favorable neurological outcomes (24).

There were several limitations to this study. CBF thresholds vary according to the rate of CBF change, which was 2.5% of the baseline level/min in our experiments; higher or lower rates could yield different thresholds for depolarization and repolarization. Various rates were tested in our pilot study, and rapid changes in CBF produced lower and higher thresholds for depolarization and repolarization, respectively; however, at any speed, thresholds were higher for membrane repolarization than for depolarization (data not shown). Ultimately, the observed rate of CBF change reflected the most stable flow control permitted by our experimental settings.

CONCLUSIONS

The measured CBF threshold for repolarization was higher than for depolarization, and this repolarization threshold may be higher than for CBF supplied by chest compressions during CPR, indicating that CPR should be initiated before the occurrence of ischemic depolarization to minimize neuronal injury. Intra-ischemic brain hypothermia decreased the CBF threshold for membrane repolarization and limited the increase in the threshold induced by prolonged ischemia, suggesting that it could assist the recovery of membrane potential during CPR and improve neurological outcome following cardiac arrest.

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Figure legends

Figure 1. Experimental procedure and groups. The extracellular potential and percent change in cerebral blood flow (CBF) in all groups are shown. Bilateral common carotid arteries were occluded (2VO) 5 min after heparin (50 U) injection; 5 min later, CBF was decreased by draining venous blood at a rate of 2.5% of the baseline value/min until a sudden negative shift in extracellular potential was observed. The CBF was maintained at a depolarized level for 5 min (Normothemia5 and Hypothermia5 groups) or 10 min (Normothermia10 and Hypothermia10 groups), and then increased by allowing venous blood to return at the same rate. The shaded region indicates the duration of selective brain hypothermia initiated immediately after depolarization (Hypothermia5 and Hypothermia10 groups).

Figure 2. Representative changes in cerebral blood flow (CBF) and extracellular potential. Arrows indicate the time of depolarization and start of repolarization. Low flow time is the period from membrane depolarization to the return of venous blood. 2VO, bilateral common carotid artery occlusion.

Figure 3. Cerebral blood flow (CBF) thresholds for membrane depolarization. There were no significant differences between groups.

Figure 4. Cerebral blood flow (CBF) thresholds for membrane repolarization. CBF thresholds were significantly higher for repolarization than for depolarization in all groups. Thresholds for repolarization were significantly lower in hypothermia than in normothermia groups (Hypothermia5 vs. Normothermia5, and Hypothermia10 vs. Normothermia10). Prolonging the low flow time increased the threshold for repolarization in normothermia but not in hypothermia groups. *$P < 0.01$ vs. depolarization, †$P < 0.01$ between groups.

Figure 5. Early initiation of high-performance CPR prevents ischemic depolarization (Zone A). Once ischemic depolarization has occurred, chest compression cannot restore the membrane potential (Zone B). Hypothermia during ischemic depolarization decreases the CBF threshold for repolarization, and chest compressions may restore the membrane potential of neuronal cells (Zone C).

Figure 1

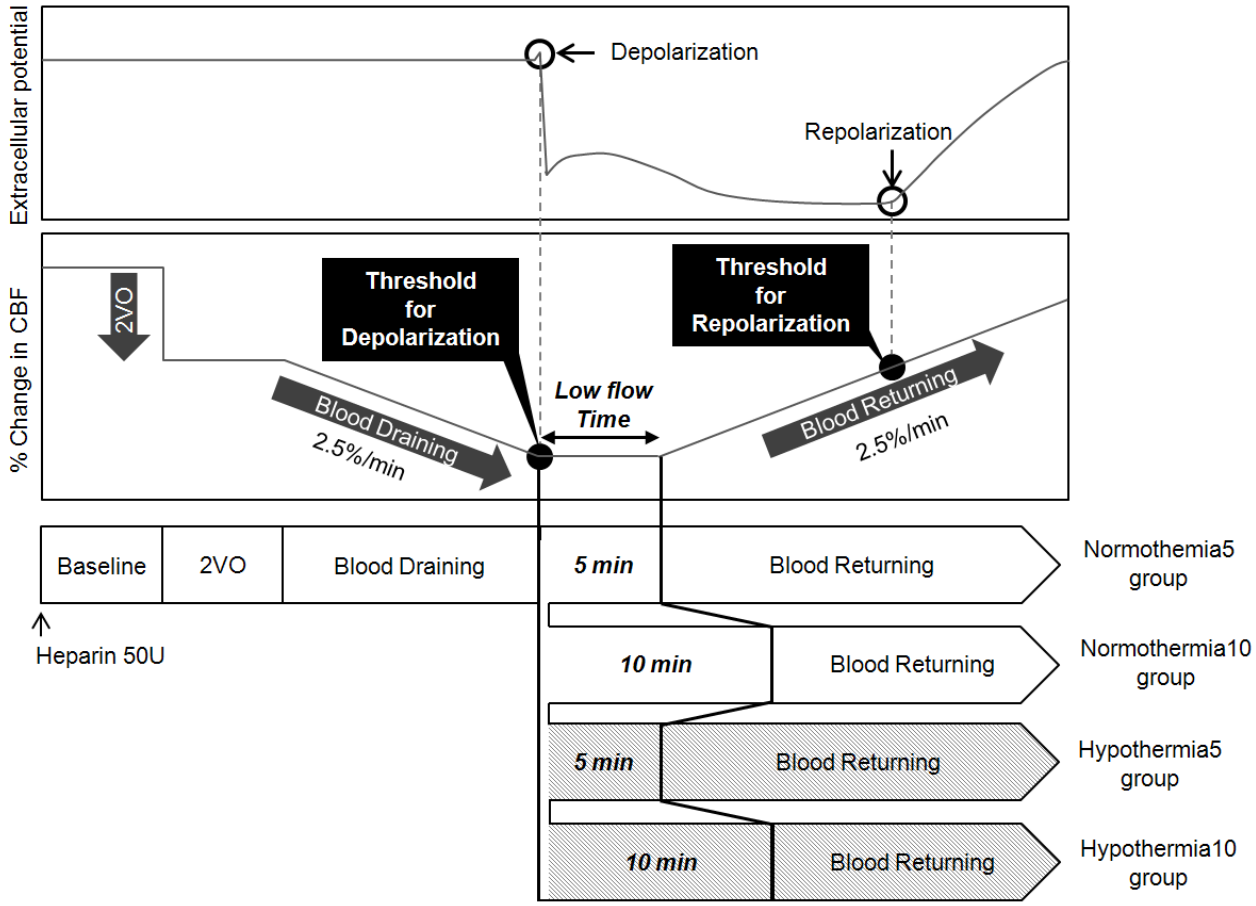

Extracellular potential
Depolarization
Repolarization
% Change in CBF
2VO
Threshold for Depolarization
Threshold for Repolarization
Low flow Time
Blood Draining 2.5%/min
Blood Returning 2.5%/min
Baseline
2VO
Blood Draining
5 min
Blood Returning
Normothemia5 group
Heparin 50U
10 min
Blood Returning
Normothermia10 group
5 min
Blood Returning
Hypothermia5 group
10 min
Blood Returning
Hypothermia10 group

Figure 2

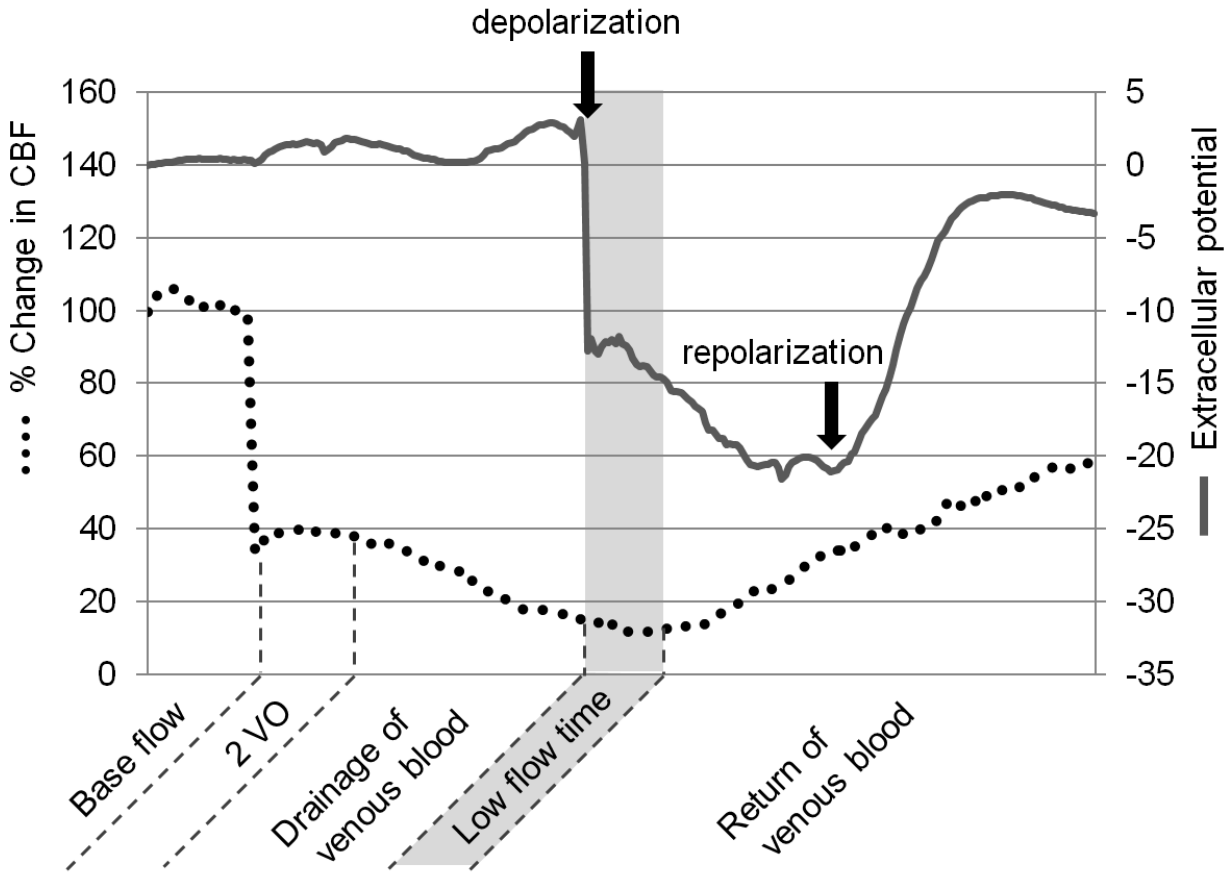

depolarization
repolarization
% Change in CBF
Extracellular potential
160
140
120
100
80
60
40
20
0
5
0
-5
-10
-15
-20
-25
-30
-35
Base flow
2 VO
Drainage of venous blood
Low flow time
Return of venous blood

Figure 3

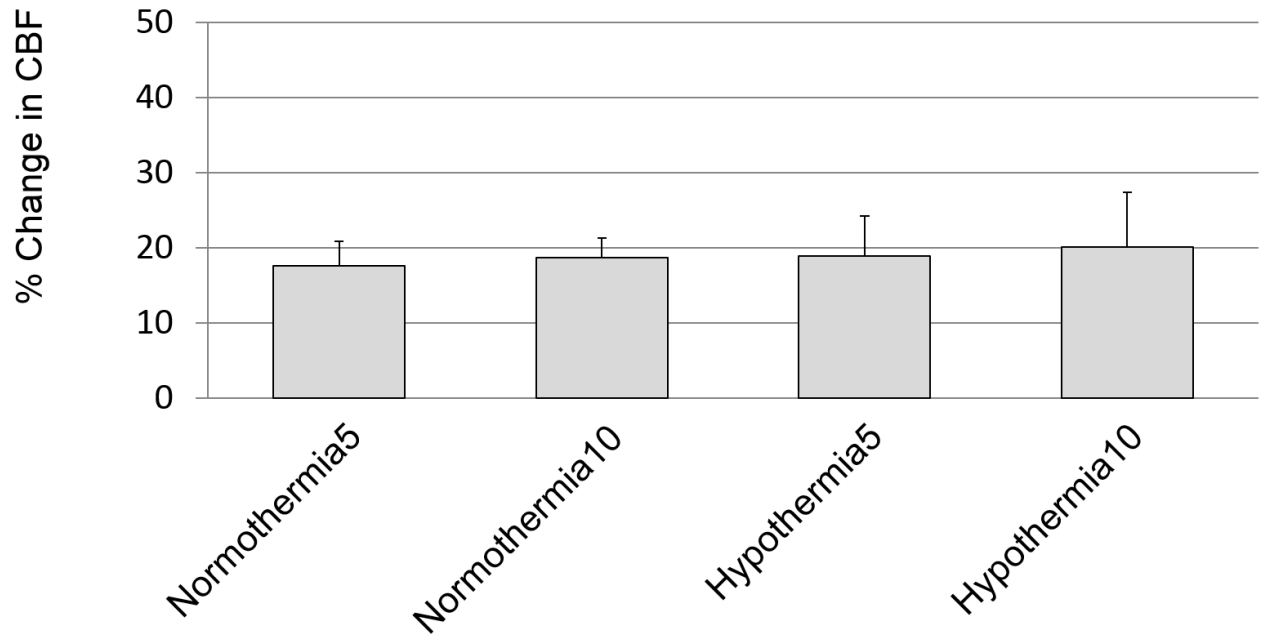

% Change in CBF
50
40
30
20
10
0
Normothermia5
Normothermia10
Hypothermia5
Hypothermia10

Figure 4

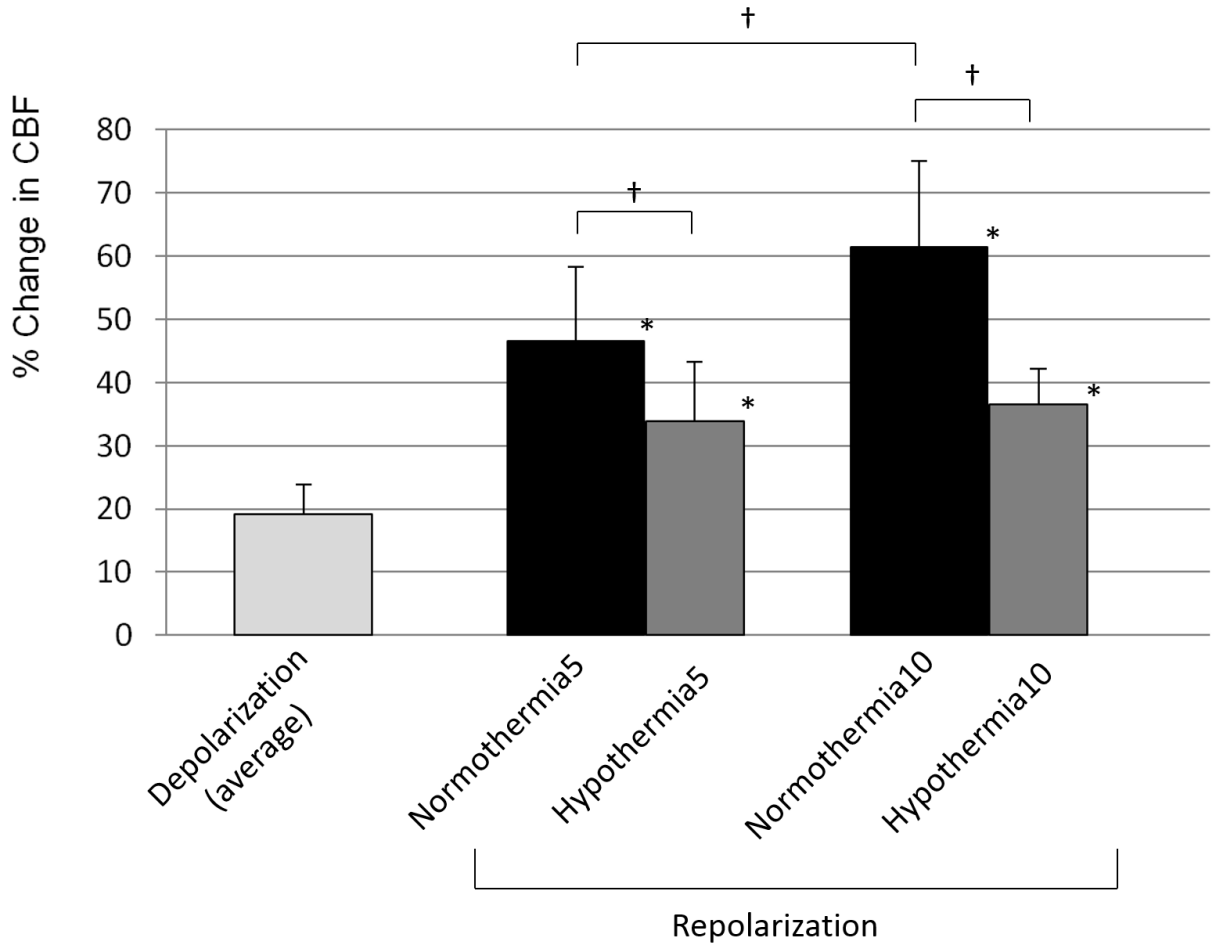
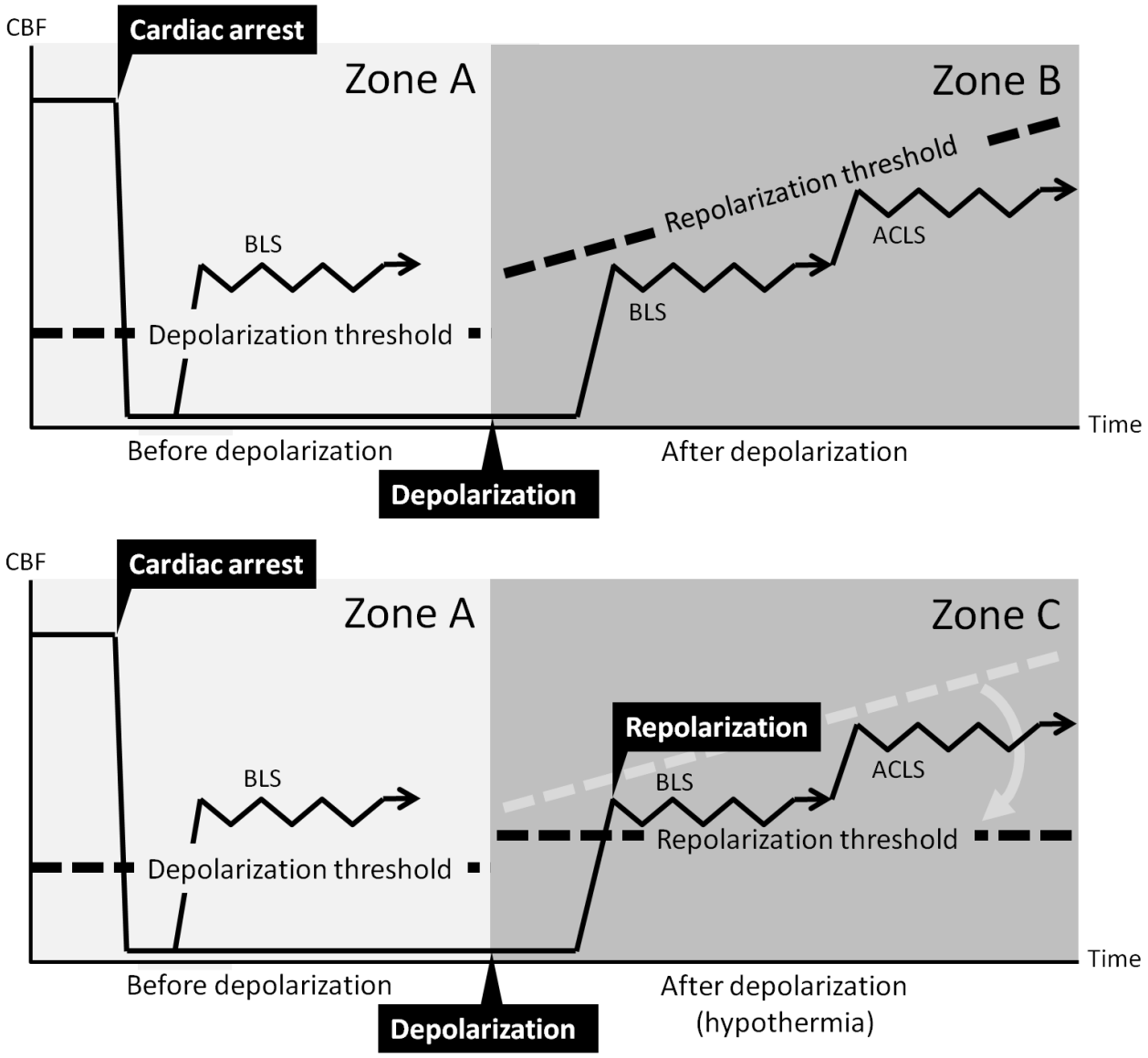

% Change in CBF
80
70
60
50
40
30
20
10
0
†
†
†
*
*
*
*
Depolarization (average)
Normothermia5
Hypothermia5
Normothermia10
Hypothermia10
Repolarization

Figure 5


CBF
Cardiac arrest
Zone A
Zone B
Repolarization threshold
ACLS
BLS
BLS
Depolarization threshold
Time
Before depolarization
After depolarization
Depolarization
CBF
Cardiac arrest
Zone A
Zone C
Repolarization
ACLS
BLS
BLS
Repolarization threshold
Depolarization threshold
Time
Before depolarization
After depolarization
(hypothermia)
Depolarization