Spontaneous postischemic hyperthermia is not required for severe CA1 ischemic damage in gerbils

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We have recently shown that brain temperature can drop even though rectal and skull readings are maintained near 37°C during global forebrain ischemia in the gerbil. In this study gerbils were subjected to 5 min of ischemia followed by 85 min of extended halothane anesthesia, while rectal and skull temperatures were kept at normal values. This extended anesthesia procedure prevented the development of spontaneous postischemic hyperthermia. However, it occasionally produced mild brain hypothermia both during ischemia and throughout anesthesia. In addition, the degree of brain hypothermia positively correlated with CA1 preservation; with some gerbils showing complete protection. In contrast, animals with normal brain temperature displayed extensive CA1 cell loss. These data suggest that postischemic hyperthermia is not a prerequisite for extensive CA1 loss in gerbils exposed to 5 min of ischemia. Second, rectal and skull recordings are not always reliable indicators of brain temperature, especially during anesthesia.

INTRODUCTION

Five min of global cerebral ischemia in the gerbil produces severe hippocampal CA1 loss. Many factors modulate this pathology, most notably brain temperature. Hypothermia either during or soon after ischemia dramatically attenuates damage. Conversely, hyperthermia aggravates ischemic damage. In relation to this observation, several authors have noted that gerbils experience transient postischemic hyperthermia of approximately 0.5–1.0°C in the gerbil. Kuroiwa and colleagues reported near complete CA1 preservation by preventing the postischemic hyperthermia associated with a 5 min occlusion in the gerbil. Postischemic temperature was assessed by skull and rectal recordings and maintained by a heating pad under extended halothane anesthesia. However, several investigators have shown that rectal temperature does not necessarily reflect brain temperature. Further, we have recently shown a dissociation between rectal, skull and brain temperatures during 5 min carotid occlusions in the gerbil. Thus, it is possible that the extended anesthesia, as used by Kuroiwa and colleagues, may foster dissociations between brain and skull/rectal temperatures.

In this study we measured brain, skull, and rectal temperatures before, during, and for several hours after 5 min of ischemia in gerbils. Some animals were kept under halothane anesthesia for 85 min after ischemia so that rectal and skull temperatures could be maintained at normal values. In this way, we assessed the effects of preventing postischemic rectal and skull hyperthermia on brain temperature and subsequent CA1 integrity.

MATERIALS AND METHODS

Subjects

Twenty female, Mongolian gerbils (High Oak Ranch Ltd., Goodwood, ON, Canada), weighing between 64 and 110 g were assigned to one of two treatment groups: control (CON; n = 7), and extended anesthesia (EXT; n = 13).

Brain temperature monitoring

Procedures for brain temperature monitoring were similar to previous work. Five days prior to occlusion gerbils had a 20 gauge guide cannula (6.0 mm) implanted at the dural surface overlying the frontal cortex. This allowed insertion of 8.0 mm brain temperature...
TABLE 1

Brain, skull, and rectal temperatures (mean ± S.D.), respectively, before (first 30–35 min of anesthesia), during and after occlusion (first 85 min of recirculation and the subsequent 3 h) in EXT and CONT animals

Values within parentheses are postanesthesia rectal recordings at 1 (under 0–85 min), 2 and 3 h (under 86–265 min), respectively.

<table>
<thead>
<tr>
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<th>PREOCCL.</th>
<th>OCCL.</th>
<th>0–85 MIN</th>
<th>86–265 MIN</th>
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<tr>
<td><strong>EXT</strong></td>
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<tr>
<td>Brain</td>
<td>36.37 ± 0.11</td>
<td>35.18 ± 1.11</td>
<td>36.23 ± 0.77</td>
<td>37.58 ± 0.44</td>
</tr>
<tr>
<td>Skull</td>
<td>36.99 ± 0.12</td>
<td>36.76 ± 0.33</td>
<td>37.14 ± 0.04</td>
<td>(37.95 ± 1.18)</td>
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<tr>
<td>Rectal</td>
<td>37.76 ± 0.40</td>
<td>37.63 ± 0.25</td>
<td>37.41 ± 0.03</td>
<td>(37.69 ± 0.73)</td>
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<tr>
<td><strong>CONT</strong></td>
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<tr>
<td>Brain</td>
<td>36.74 ± 0.09</td>
<td>35.00 ± 1.06</td>
<td>37.31 ± 0.89</td>
<td>37.94 ± 0.73</td>
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<tr>
<td>Skull</td>
<td>37.18 ± 0.17</td>
<td>36.87 ± 0.35</td>
<td></td>
<td>(37.63 ± 0.78)</td>
</tr>
<tr>
<td>Rectal</td>
<td>37.87 ± 0.24</td>
<td>37.59 ± 0.24</td>
<td>(37.06 ± 0.65)</td>
<td>(38.43 ± 0.93)</td>
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Two days prior to ischemia gerbils were briefly anesthetized with 2.0% halothane (30% O2 and 70% N2O) while the brain probes were inserted. Following insertion anesthesia was discontinued and the gerbils were placed into small plexiglass cages resting on FM receivers (Mini-Mitter, Model RA-1010). Brain temperature was recorded continuously for approximately 3 h to provide baseline data on normal brain temperature. Temperature data were analyzed and plotted using Dataquest III software (Data Sciences, Inc., St. Paul, MN, USA).

Cerebral ischemia

Two days after baseline temperature was recorded, the gerbils were anesthetized with 1.5–2.0% halothane in 30% O2 and 70% N2O. Rectal temperature was monitored and maintained near 37.5°C with a homeothermic blanket unit (Harvard Apparatus, South Natick, MA, USA). Skull temperature was measured by inserting a 30 gauge thermocouple probe (Omega Engineering, Stamford, CT, USA) subcutaneously on the opposite side of the guide cannula. Skull temperature was held near 37.0°C by a heated water flow-through blanket (Mul-T-Pads, Model TP-3E, Gaymar Industries, Inc., Orchard Park, NY, USA) which was in direct contact with the head. Skull temperature was continuously monitored to ensure steady temperature control.

A small opening in the water blanket permitted passage of the cannula into which the brain probe was inserted. This allowed brain temperature recording without interference with the water blanket. Once all probes were in place the common carotid arteries were isolated and exposed. After 30–35 min of anesthesia the arteries were occluded for 5 min. Both the occlusion and subsequent anesthesia were performed under 1.0% halothane. In CONT animals anesthesia was discontinued and gerbils, with brain probes in place, were placed in cages resting on the FM receivers. Brain temperature was then monitored for 4 h and 25 min. In the EXT group, gerbils were maintained with 1.0% halothane for 85 min after occlusion while their skull and rectal temperatures were kept at normal and their brain temperature was monitored. After 85 min, anesthesia was discontinued and gerbils were placed in cages resting on the FM receivers for 3 more hours of continuous brain temperature recording.

Rectal temperature (Digi-Sense, Cole-Parmer Instrument Company, Chicago, IL, USA) was recorded 1, 2, and 3 h after ischemia in...
Fig. 3. CA1 preservation in a hypothermic gerbil (A) and severe CA1 necrosis in a representative gerbil (B) who was normothermic in the peri-ischemic period. The respective temperature profiles of these animals are shown in Figs. 1 and 2.
CONT animals. In EXT gerbils, rectal temperature was simultaneously recorded (blanket unit) with skull values during the 85 min of postischemic anesthesia. Rectal temperature was then sampled with the Digi-Sense probe 2 and 3 h after ischemia.

Histology

Gerbils were sacrificed 10 days after ischemia. Ten μm coronal tissue slices were collected and analyzed as described previously. Briefly, the medial, middle and lateral sectors of the CA1 pyramidal cell layer, at a level approximately 1.7 mm posterior to bregma, were rated by two experimentally naive observers. The percentage of normal neurons was rated on a 5 point scale as follows: 0 = 0–5%, 1 = 6–29%, 2 = 30–59%, 3 = 60–89%, and 4 = 90–100%. A total score of 24 (3 sectors per hemisphere) indicated a normal CA1. Previous data (n = 56, unpublished data) has shown that this rating score correlates highly with CA1 cell counts, r = 0.973, P = 0.0001. In addition, both our rating and counting methods include only viable-looking neurons (i.e., well-defined nuclei).

RESULTS

Normal brain temperature, recorded 2 days prior to occlusion, was 37.08 ± 0.50 S.D., similar to our previous findings. The degree of spontaneous postischemic hyperthermia in CONT animals (peak brain temperature = 37.88°C at 35 min postischemia, and mean brain temperature of the first 85 min after occlusion = 37.31°C) was also similar to previous work. Maintaining postischemic skull and rectal normothermia, under halothane anesthesia, prevented this temperature rise (Table I). Regardless, the average CA1 ratings (out of 24) were almost identical at 7.08 ± 7.60 S.D. and 6.00 ± 5.51 S.D. in the EXT and CONT groups, respectively. Cell damage ranged from 0 (completely damaged) to 23 (no damage).

In spite of rectal and skull normothermia (Table I) several animals, in both groups, displayed significant brain hypothermia. In animals that experienced normothermic (brain) ischemia there was severe CA1 cell death, whereas animals that experienced prolonged cerebral hypothermia during and/or after ischemia had reduced CA1 damage (Figs. 1–3).

Mean brain temperature (n = 20), during occlusion, was significantly correlated with histological damage, r = −0.587, P = 0.029. Mean brain temperature (n = 20) for the initial 85 min of reperfusion was also statistically related to histological outcome, r = −0.558, P = 0.011. Thus, it appears that mild brain hypothermia either during ischemia or in the first 85 min of reperfusion affords histological protection. However, mean brain temperature (n = 20) averaged over a 3 h period starting 86 min after ischemia was not related to histological outcome, r = −0.120, P = 0.613. Since rectal and skull temperatures were normothermic during anesthesia there was no meaningful relationship between these and histological outcome. There was also no significant relationship between postanesthetic rectal readings and CA1 rating (statistics not shown).

DISCUSSION

There was marked CA1 cell loss in both the EXT and CONT groups, however, the variability was somewhat high. Our recent data indicate that more severe and consistent CA1 necrosis is produced with regulation of brain rather than skull temperature. However, in this study we attempted to approximate the procedures used by Kuroiwa et al. who did not measure brain temperature.

This experiment shows that brain temperature can markedly dissociate from both skull and rectal readings during ischemia surgery with lengthened anesthesia in the gerbil. We also show that 5 min of ischemia in those EXT gerbils with an intraischemic brain temperature range of 35.62–36.78°C, and a postischemic range of 36.48–37.25°C results in severe CA1 necrosis (2.83 ± 2.48 S.D., n = 6). This result contrasts with that of Kuroiwa, Bonnekoh and Hossmann, where they found robust CA1 preservation by preventing the spontaneous postischemic rise in temperature normally associated with 5 min occlusions in gerbils. They used various durations of extended halothane anesthesia (up to 85 min) and a rectal heating pad to maintain rectal and skull normothermia during and after ischemia. However, brain temperature was not assessed. Importantly, we observed brain hypothermia, with skull and rectal normothermia, in several animals, which was quite dramatic in 2 of the 13 EXT gerbils (Fig. 1). Perhaps Kuroiwa et al. also induced protracted mild brain hypothermia in many of their extended anesthesia animals. This brain hypothermia, rather than prevention of postischemic hyperthermia, may have afforded CA1 protection in their study. Without brain temperature data, they cannot conclude that postischemic hyperthermia, as compared to normothermia, is required for CA1 damage. We may not have encountered as many gerbils with such dissociations because our heating system utilizes rectal and skull blankets rather than a single heating pad. We used the cranial blanket since the brain probe elevates the head above the body blanket, thereby reducing its effectiveness. Our heating system would likely reduce the incidence of brain temperature dissociations.

The degree of postischemic brain hyperthermia in CONT animals was less than the approximately 1.5°C rise in rectal and skull temperatures noted by Kuroiwa et al. However, the degree of postischemic hyperthermia observed in the present study is comparable to other findings in the gerbil. Perhaps the greater
postischemic rise in their study was partially due to the stress of taking multiple rectal and skull readings. Our brain temperature system does not cause any noticeable stress and, therefore, would not likely alter temperature.

In conclusion, 5 min of normothermic ischemia followed by 85 min of normothermic recirculation resulted in severe CA1 necrosis. Thus, spontaneous postischemic hyperthermia was not necessary for severe CA1 damage. This does not indicate that postischemic hyperthermia is not important. It is likely that greater temperature rises soon after ischemia would aggravate damage, such as through recruiting additional brain regions to necrosis. For example, Mitani and Kataoka\(^{11}\) showed that elevation of brain temperature to 39°C was associated with a prolonged rise in glutamate levels and increased CA1 cell loss. Finally, our study shows that prolonged mild brain hypothermia may occur with extended anesthesia, resulting in histological protection. These results and our previous study\(^{4}\) suggest that brain temperature should be determined in any ischemia study, especially those utilizing extended periods of anesthesia or other procedures that might promote skull/rectal and brain temperature dissociations.

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REFERENCES

5 Hewitt, K.E. and Corbett, D., Combined treatment with MK-801 and nicardipine reduces global ischemic damage in the gerbil, Stroke, 23 (1992) 82–86.
7 Kirino, T., Delayed neuronal death in the gerbil hippocampus following ischemia, Brain Res., 237 (1982) 57–69.