The Arctic Ground Squirrel Brain Is Resistant to Injury From Cardiac Arrest During Euthermia

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Kelly L. Drew, PhD; Miguel A. Perez-Pinzon, PhD

Background and Purpose—Heterothermic mammals tolerate hypoxia during euthermia and torpor, and evidence suggests this tolerance may extend beyond hypoxia to cerebral ischemia. During hibernation, CA1 hippocampal neurons endure extreme fluctuations in cerebral blood flow during transitions into and out of torpor as well as reductions in cerebral blood flow during torpor. In vitro studies likewise show evidence of ischemia tolerance in hippocampal slices harvested from euthermic ground squirrels; however, no studies have investigated tolerance in a clinically relevant model of in vivo global cerebral ischemia. The purpose of the present study was to test the hypothesis that the euthermic Arctic ground squirrel (AGS; Spermophilus parryii) is resistant to injury from asphyxial cardiac arrest (CA).

Methods—Estrous-matched female rats were used as a positive control. Female euthermic AGS and rats were subjected to 8-minute CA. At the end of 7 days of reperfusion, AGS and rats were fixed for histopathological assessment.

Results—In rats subjected to CA, the number of ischemic neurons was significantly higher \( P < 0.001 \) compared with control rats in hippocampus and striatum. Cortex was mildly injured. Surprisingly, neuronal counts in AGS were not significantly different in CA and control groups in these brain regions.

Conclusion—These data demonstrate that AGS are remarkably tolerant to global cerebral ischemia during euthermia. A better understanding of the mechanisms by which AGS tolerate severe reductions in blood flow during euthermia may provide novel neuroprotective strategies that may translate into significant improvements in human patient outcomes after CA. (Stroke. 2006;37:1261-1265.)

Key Words: cerebral ischemia | heart arrest | neuroprotection

Cardiopulmonary arrest and stroke remain 2 of the leading causes of death and disability in the United States. It is now understood that the mechanisms leading to neuronal cell death after cardiac arrest (CA) or stroke are highly complex. A well-established fact in this field is that neurons continue to die over days and months after ischemia. In the case of CA, the chances of survival are poor, despite fast emergency responses and better techniques of defibrillation. In fact, from the \( \approx 70,000 \) patients per year who are resuscitated after CA, 60% die from extensive brain injury, and only 3% to 10% are able to resume their former lifestyles.\(^1\) Thus, development of novel therapies to protect the brain from the ravages of cerebral ischemia is key to improvement in survival and better outcomes after such a devastating condition.

One potential source for the development of novel therapies comes from the study of heterothermic mammals (mammals that hibernate). Heterothermic mammals in the nonhibernating (euthermic) state survive hypoxia better than nonhibernating species.\(^2\) This tolerance has been suggested to have evolved as a mean to tolerate transitions into and out of torpor.\(^3\) The Arctic ground squirrel (AGS; \( S \) parryii) for example, tolerates a 50% decrease in oxygen–hemoglobin saturation during arousal with arterial oxygen partial pressure reaching as low as 7 mm Hg without evidence of damage to hippocampal CA1 or cortical neurons.\(^2,3\) The normal course of hibernation similarly gives rise to extreme fluctuations in cerebral blood flow, reaching near–ischemic-like levels during torpor and returning in a reperfusion-like manner during arousal.\(^4\) Tolerance to low blood flow in the hibernating state is likely facilitated by metabolic suppression and other aspects of the hibernation phenotype.\(^5\) However, tolerance to fluctuations in blood flow during entrance and emergence from torpor suggests heterothermic mammals may also tolerate ischemia in the euthermic state. This hypothesis is supported by in vitro data in which hippocampal slices from euthermic ground squirrels tolerate oxygen glucose deprivation better than slices from rat.\(^11\) However, it is unclear whether these in vitro observations translate to the in vivo...
condition. Therefore, the purpose of the present study was to assess tolerance to global ischemia using a clinically relevant model of asphyxia-induced CA in euthermic AGS.

Methods

Cardiac Arrest

All animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and approved by the animal care committees of the University of Miami (UM) and the University of Alaska Fairbanks (UAF). Wild AGS were trapped in mid-July at \( \sim 68^\circ\)W, transported to UAF, and quarantined and housed at \( 17\)°C under natural lighting conditions and until September. AGS were then housed at \( 2^\circ\)C and a 4-hour light/24-hour dark cycle from September to May and hibernated normally. In May, AGS were moved to a warm room (\( 17^\circ\)C) and a 12-hour light/dark cycle, and in July, they were flown to UM where they were housed under similar conditions (\( 17^\circ\)C and a 12-hour light/dark cycle). Food was available ad libitum at all times. All animals remained euthermic at UM, and experiments were conducted during late August and early September. Female AGS were used for the present study because of availability of wild trapped animals at the time of the study. Female Sprague-Dawley rats were purchased from Charles River Laboratories, Wilmington, Mass. Estrous-matched female AGS (\( n=5\)) and female Sprague-Dawley rats (\( n=8\)) weighing 63 to 84 g and 245 to 275 g, respectively, were used for the study. Procedures for induction of CA and resuscitation were described previously. After 10 minutes of restoration of spontaneous circulation (ROSC), the ventilator rate was decreased to 60 breaths per minute and the diastolic blood pressure was performed using repeated-measures ANOVA followed by multiple-comparison procedure (Student-Newman-Keuls test) to isolate group differences. Statistical evaluation of the data was performed using ANOVA followed by Bonferroni’s post hoc test.

Histology

After 7 days of reperfusion, AGS and rats were perfused with FAM (a mixture of 40% formaldehyde, glacial acetic acid, and methanol, 1:1:8 by volume) for 19 minutes after a 1-minute initial perfusion with physiological saline. The perfusate was delivered into the root of the ascending aorta at a constant pressure of 110 to 120 mm Hg as described previously. The head was removed and immersed in FAM at 4°C for 1 day. The brain was then removed from the skull, and coronal brain blocks were embedded in paraffin; coronal sections of 10 \( \mu \)m were stained with hematoxylin and eosin. In the rats, sections containing hippocampus at the level of 3.8 mm posterior to bregma were examined, whereas dorsolateral striatum at the level of 1.8 mm posterior to bregma was examined. In AGS, equivalent areas were examined. Normal neuronal counts were made within the CA1 region of hippocampus by an investigator blinded to the experimental conditions and the counts are expressed as number of normal neurons present in entire CA1 region. For dorsolateral striatum the number of ischemic neurons were counted because the number of ischemic/ghost neurons were much lower than normal-appearing neurons. The number of ischemic neurons expressed here are per microscopic field (\( \times 100 \) magnification). We did not count ischemic or normal neurons in the cortex because in rats subjected to CA, cortical pathology was very subtle and mild; however, AGS did not exhibit any neuropathology.

Statistics

All data are expressed as mean±SD. Statistical evaluation of the data of heart rate, mean arterial blood pressure, systolic blood pressure, and diastolic blood pressure was performed using repeated-measures ANOVA followed by multiple-comparison procedure (Student-Newman-Keuls test) to isolate group differences. Statistical evaluation of the ascending aorta at a constant pressure of 110 to 120 mm Hg as described previously. The head was removed and immersed in FAM at 4°C for 1 day. The brain was then removed from the skull, and coronal brain blocks were embedded in paraffin; coronal sections of 10 \( \mu \)m were stained with hematoxylin and eosin. In the rats, sections containing hippocampus at the level of 3.8 mm posterior to bregma were examined, whereas dorsolateral striatum at the level of 1.8 mm posterior to bregma was examined. In AGS, equivalent areas were examined. Normal neuronal counts were made within the CA1 region of hippocampus by an investigator blinded to the experimental conditions and the counts are expressed as number of normal neurons present in entire CA1 region. For dorsolateral striatum the number of ischemic neurons were counted because the number of ischemic/ghost neurons were much lower than normal-appearing neurons. The number of ischemic neurons expressed here are per microscopic field (\( \times 100 \) magnification). We did not count ischemic or normal neurons in the cortex because in rats subjected to CA, cortical pathology was very subtle and mild; however, AGS did not exhibit any neuropathology.

Physiological Parameters

<table>
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<tr>
<th>Group</th>
<th>Variable</th>
<th>Ischemia</th>
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<th>After</th>
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<tr>
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<td>693±111</td>
<td>693±111</td>
<td>693±111</td>
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<td></td>
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<td></td>
<td>HCO&lt;sub&gt;3&lt;/sub&gt; mmol/L</td>
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<tr>
<td></td>
<td>Plasma glucose mg/dL</td>
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*P<0.05 rat vs AGS; #P<0.05 baseline vs postarrest.

Results

To test the hypothesis that AGS are ischemia tolerant during euthermia, we first characterized the basic physiological parameters between the 2 species. For these studies, the mean body weights of AGS and rats were 693±111 and 253±9 g, respectively. In rats, the mean percentage of hematocrit was higher compared with the AGS (AGS 44±4.5 versus rats 47±1.0). Because it is customary in our studies of CA, both species were fasted overnight before experiments. However, in spite of overnight fasting, the mean plasma glucose in AGS was higher by 56% compared with rats (Table). Another physiological parameter that exhibited marked differences between the 2 species was blood gases. In rats the blood P<sub>O2</sub> and P<sub>CO2</sub> levels before induction of CA were 119±32 and 39±4 mm Hg, respectively (Table). In contrast, blood P<sub>O2</sub> and P<sub>CO2</sub> levels in AGS before induction of CA were 49±10 and 60±4 mm Hg, respectively, consistent with normal values reported for this species. During resuscitation, rats and AGS were mechanically ventilated with 100% oxygen. Thus, in rats 10 minutes after resuscitation, P<sub>O2</sub> increased by 25% compared with baseline (before CA). In contrast to rats, 10 minutes after resuscitation, P<sub>O2</sub> was lower by 27% in AGS. Despite the differences in plasma P<sub>O2</sub> and P<sub>CO2</sub> levels in both species, plasma pH was similar in both species (AGS 7.46±0.04 versus rat 7.48±0.06; Table).

In both species, an immediate bradycardia was observed when apnea was induced during the induction of CA, followed by hypotension to 50 mm Hg. Within \( \sim 3 \) to 4 minutes, mean arterial pressure decreased to 0 mm Hg, and the heart rate was further decreased (Figure 1). Systolic and diastolic pressures decreased in parallel in both species. On resuscitation, mean arterial pressure returned to 60 mm Hg within 2 minutes in both species (Figure 1). The ECG pattern was restored to normal within 5 minutes of ROSC in both species.

In rats, 6 of 8 animals survived for 7 days after ROSC. In AGS, all 5 animals survived for 7 days. The mean body temperature in rats subjected to CA was 37.1±0.20, 36.58±0.58, and 36.33±0.41 at 24, 48, and 72 hours after resuscitation, respectively. The mean body temperature in AGS subjected to
CA was 36.7±0.45, 36.95±0.27, and 36.85±0.60 at 24, 48, and 72 hours after resuscitation, respectively. We did not observe any torpor/hypothermia in AGS after CA. Although we did not carry out any neurological assessment in these studies, we observed a pronounced difference in the recovery of these species to ROSC. In rats, postresuscitation recovery was slow and characterized by an inability to care for themselves, which required supportive care consisting of gavage feeding (crushed rat chow in water) for 72 hours. In contrast, AGS were drinking and feeding immediately after ROSC.

Histological assessment of neuronal death in the brain was performed 7 days after 8 minutes of CA (Figure 2). In rats, neurons exhibiting ischemic cell change were present throughout the forebrain and hindbrain. These changes consisted of eosinophilic cytoplasm, dark-staining triangular-shaped nucleus, and eosinophilic-staining nucleolus. In rats, the number of normal neurons in the CA1 hippocampal region at 7 days after sham-arrest and 8 minutes CA were 941±56 and 230±100, respectively. In the AGS, the numbers of normal neurons were statistically similar in sham-operated and CA groups (sham 900±134 versus CA 904±61; Figure 3A).

The number of ischemic neurons in the dorsolateral striatum of rats subjected to sham operation and CA was 5±1 and 67±40, respectively (P<0.01 sham versus CA; Figures 2 and 3B). In the dorsolateral striatum of AGS, the numbers of ischemic neurons were statistically similar in sham and CA groups (sham 5±2 versus CA 7±2; Figures 2 and 3B). In all cortical layers of rat cortex subjected to CA, scattered mild and subtle neuronal death was observed. However, in the cortex of AGS subjected to CA, we did not observe any neuronal death (Figure 2).

**Discussion**

Here we show for the first time unparalleled tolerance to cerebral ischemia in a mammal. Using a clinical model of
CA, we demonstrate that euthermic AGS is resistant to injury from an ischemic insult even when the mean arterial blood pressure fell to 0 rapidly on initiation of the insult, and all physiological parameters were controlled, especially temperature, which is a well-known neuroprotectant against ischemic damage. Our findings are of particular importance because AGS ischemic tolerance occurred during euthermia rather than during torpor. Although multiple adaptations including metabolic suppression would be expected to contribute to ischemia tolerance during torpor, tolerance in the euthermic state may involve mechanisms that are more readily attainable in nonhibernating species, such as humans in which morbidity attributable to CA and stroke remains a serious health problem. Previous support for ischemic tolerance of AGS was demonstrated in vitro by Frerichs and Hallenbeck, who measured CA1 neuronal cell death in hippocampal slices harvested from hibernating and euthermic ground squirrels and rats at 7°C to 36°C. They found that slices from active ground squirrel were more tolerant than rats at 20°C and 7°C but not at 36°C, indicating a species-specific difference that becomes manifest at lower temperatures. More recent in vitro studies in AGS show that slices from euthermic AGS tolerate oxygen glucose deprivation better than rats, even at 37°C.

Mechanisms of ischemia tolerance in AGS may be related to adaptations associated with evolution of heterothermy. Currently, there is controversy as to whether low cerebral blood flow during torpor constitutes ischemia because neither an energy deficit nor neuronal pathology ensues during hibernation. It is clear that these species have the ability to titrate supply and demand for cerebral blood flow and that this is an essential feature of successful hibernation. Although a rapid suppression of metabolic demand in response to ischemia may contribute to ischemia tolerance in the euthermic state, and warrants further investigation, metabolic suppression is unlikely to account for the full degree of tolerance observed in the present study. In contrast to what is observed during fluctuations in cerebral blood flow during the natural course of hibernation, euthermic AGS suffered acutely from the initial ischemic insult. These animals required resuscitation similar to rats immediately after CA. However, postresuscitation recovery was more rapid and was not associated with neuronal cell death.

A clue to the potential mechanism of ischemia tolerance arises from the fact that euthermic AGS maintained lower arterial PO2 and elevated PCO2 compared with rats. In fact, we observed early on in our studies during the development of the AGS CA model that when AGS arterial PO2 and PCO2 were adjusted to levels similar to those found in rats, AGS developed hemodynamic perturbations characterized by hypotension, and when forced to undergo CA under these conditions, these AGS could not be resuscitated (data not shown). Interestingly, the low PO2 levels found in AGS during euthermia is accompanied by increased hypoxia-inducing factor–1α protein levels in brain, suggesting this species experiences mild, chronic hypoxia attributable to low respiratory drive. Hypoxia-induced preconditioning is linked to hypoxia-inducing factor–1α–regulated gene expression that enhances tolerance to subsequent ischemic events. Protective effects of hypoxic preconditioning are reported using in vivo and in vitro models of cerebral ischemia. Tolerance to global cerebral ischemia in euthermic AGS may thus be associated with a preconditioning effect stemming from a chronically low respiratory drive. Higher arterial PCO2 in euthermic AGS compared with rats also suggests mild acidification in the AGS brain. Tang et al showed that increased external [H+] suppresses N-methyl-D-aspartate (NMDA)–activated current. This suggests that NMDA receptors were suppressed in AGS brain at the time of CA, which may be responsible for lower excitotoxicity and thus neuroprotection.

Finally, seasonal adaptations in preparation of the hibernation season may contribute to the neuroprotection observed in AGS in the present study. Experiments were conducted in August, when female AGS prepare to hibernate. Seasonal changes in antioxidant defense and immune responsiveness have been described in AGS and estivating species. In addition, a subset of heterotherms, including AGS, are fossorial, and fossorial animals display hypoxia tolerance. However, it has yet to be shown whether fossorial animals in general tolerate ischemia as well as hypoxia. Evidence from other hypoxic-tolerant species suggests that some of the adaptations are similar but perhaps not sufficient to promote ischemic tolerance. For example, turtle brain is highly resistant to anoxia, but inhibition of glycolysis (as it would occur during ischemia) renders this species highly vulnerable to injury.

Summary

In conclusion, the present results demonstrate a remarkable tolerance to CA in AGS during euthermia when brain temperature was maintained at 37°C. Future studies will be necessary to determine the maximal duration of CA tolerated by this species and to define the mechanisms by which these species become ischemic tolerant. A better understanding of the mechanisms by which AGS tolerate severe reductions in blood flow during euthermia may provide novel neuroprotective strategies that may translate into significant improvements in human patient outcomes after CA.

Acknowledgments

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References


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