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THERAPEUTIC HYPOTHERMIA PROTECTS AGAINST ISCHEMIA-INDUCED IMPAIRMENT OF SYNAPTIC PLASTICITY FOLLOWING JUVENILE CARDIAC ARREST IN SEX-DEPENDENT MANNER

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Abstract—Pediatric cardiac arrest (CA) often leads to poor neurologic outcomes, including deficits in learning and memory. The only approved treatment for CA is therapeutic hypothermia, although its utility in the pediatric population remains unclear. This study analyzed the effect of mild therapeutic hypothermia after CA in juvenile mice on hippocampal neuronal injury and the cellular model of learning and memory, termed long-term potentiation (LTP). Juvenile mice were subjected to cardiac arrest and cardiopulmonary resuscitation (CA/CPR) followed by normothermia (37 °C) and hypothermia (30 °C, 32 °C). Histological injury of hippocampal CA1 neurons was performed 3 days after resuscitation using hematoxylin and eosin (H&E) staining. Field excitatory post-synaptic potentials (fEPSPs) were recorded from acute hippocampal slices 7 days after CA/CPR to determine LTP. Synaptic function was impaired 7 days after CA/CPR. Mice exposed to hypothermia showed equivalent neuroprotection, but exhibited sexually dimorphic protection against ischemia-induced impairment of LTP. Hypothermia (32 °C) protects synaptic plasticity more effectively in females, with males requiring a deeper level of hypothermia (30 °C) for equivalent protection. In conclusion, male and female juvenile mice exhibit equivalent neuronal injury following CA/CPR and hypothermia protects both males and females. We made the surprising finding that juvenile mice have a sexually dimorphic response to mild therapeutic hypothermia protection of synaptic function, where males may need a deeper level of hypothermia for equivalent synaptic protection.

Key words: hypothermia, cardiac arrest, global ischemia, pediatric, synaptic plasticity, synaptic function.

Cardiac arrest (CA) is an important and tragic event that has high morbidity across all ages. It is estimated that 600,000 adults and 16,000 children per year suffer CA in the United States (Sirbaugh et al., 1999; Michiels et al., 2013), leading to significant global ischemia and neurologic injury in those who survive. For reasons that are unclear, boys are at greater risk of CA than girls (Sirbaugh et al., 1999). The sequelae of pediatric CA result in a lifetime of dependency for all aspects of care (Sirbaugh et al., 1999; Moler et al., 2011; Michiels et al., 2013). A great deal of research has focused on improving these outcomes through efforts such as neuroprotection and improved rates of return of spontaneous circulation (ROSC). To date, therapeutic hypothermia is the only therapy shown to be effective in increasing survival and improving neurologic outcome and has become the standard of care in adults and neonates less than 6 h old (Arrich et al., 2012; Jacobs et al., 2013). However, a recent study of hypothermia in children with out-of-hospital CA showed no effect on survival with good neurobehavioral outcome (Moler et al., 2015). Differences in the etiology and pathophysiology may exist among different age groups and neuroprotective strategies following CA in neonates and adults may not be generalizable to children. In fact, little experimental data exist to assess the differences in brain injury following CA in adults versus children. We recently established a juvenile model of cardiac arrest and cardiopulmonary resuscitation (CA/CPR) (Deng et al., 2014) to begin addressing these differences. We found that there is similar neuronal injury between juvenile and adult male mice and that hypothermia decreased neuronal death (Deng et al., 2014). The current study is designed to assess the impact of hypothermia on neuronal injury and synaptic functional recovery in the young brain following CA/CPR.

Despite much effort, the field of cerebral ischemia has been plagued by lack of translatable strategies, even though there has been much promise in the laboratory in delivering neuroprotection (Herson and Traystman, 2014). This highlights the importance of moving beyond focusing on neuronal protection and, in turn, identifying

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Abbreviations: aCSF, artificial cerebral spinal fluid; AP5, D-2-amino-5-phosphonopentanoate; CA, cardiac arrest; CA/CPR, cardiac arrest and cardiopulmonary resuscitation; fEPSPs, field excitatory post-synaptic potentials; FiO_2, fraction of inspired oxygen; H&E, hematoxylin and eosin; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; PPR, paired-pulse ratio; ROSC, return of spontaneous circulation; TBS, theta burst stimulation.

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approaches to improve functional recovery following cerebral ischemia. The ability of neurons to undergo synaptic plasticity (long-term potentiation; LTP) is recognized as an innate measure of function and is a widely accepted cellular model for learning and memory (Bliss and Collingridge, 1993; Neves et al., 2008). Hippocampal CA1 pyramidal neurons have been well studied in synaptic plasticity and are particularly susceptible to ischemic injury (Schmidt-Kastner and Freund, 1991), making these neurons useful targets in studying functional injury after ischemia. Ischemia-induced impairment of LTP is well established in adult models of ischemia, and many have implicated the loss of *N*-methyl-D-aspartate (NMDA) receptor expression and function (Zhang et al., 1997; Liu et al., 2010), though this remains an open question (Orfila et al., 2014). Surprisingly, little is known about the effect of ischemia on synaptic plasticity in the young brain or the effects of hypothermia on synaptic function following ischemia (Miyamoto et al., 2000). Here, we present the first data showing that a hypothermia strategy can preserve synaptic function after juvenile CA/CPR with sex-specific efficacy.

EXPERIMENTAL PROCEDURES

Experimental animals

All experimental protocols were approved by the Institutional Animal Care and Use Committee and conformed to the National Institutes of Health guidelines for care and use of animals. Male and female C57Bl/6 20–25-day-old (P20-25) mice (Charles River Laboratory) were used for this study. P20-25 mice are prepubertal mice and reported to be equivalent to 2–4-year-old children (Semple et al., 2013) and all analysis was completed by day 32, equivalent to less than 11-year-old children (Semple et al., 2013). These mice were weaned and not with dam at the time of experiment. The mice were housed in a standard 12-h light and 12-h dark cycle and had free access to food and water. Mice were randomly assigned to experimental groups and the investigator was blinded. A total of 66 mice were used for this study. The average age at CA/CPR was 23.2 ± 0.2 day old (n = 51). The average age for mice that were sacrificed for histology was 26.6 ± 0.2 days old (n = 26) and the average age for mice sacrificed for electrophysiology was 29.5 ± 0.3 days old (n = 22). Three mice were removed from the study due to premature death after CA/CPR (1 male in 37 °C group, 1 female in 32 °C group and 1 male in the 32 °C group). One additional mouse was removed in the male 32 °C group due to lack of usable signals. Age matched controls for electrophysiology averaged 28.8 ± 0.5 days old (n = 15).

CA/CPR

CA in juvenile mice was performed as previously described (Deng et al., 2014). Briefly, mice were anesthetized using 3% isoflurane and maintained with 1.5–2% isoflurane in 30% fraction of inspired oxygen (FiO_2) via face mask. Body temperature was maintained at 37 °C using a heat lamp and heating pad while being monitored with temperature probes placed into the left ear canal and rectum. For drug administration, a PE-10 catheter was inserted into the right internal jugular vein and flushed with heparinized 0.9% normal saline solution. Animals were endotracheally intubated using a 24G intravenous catheter and connected to a mouse ventilator (Minivent, Hugo Sachs Elektronik, March-Hugstetten, Germany) set to a respiratory rate of 160 breaths per minute. Cardiac function was monitored throughout the experiment with EKG. CA was induced by injection of 30 μL of 0.5 M KCl via the jugular catheter and confirmed by asystole on electrocardiography and absence of spontaneous breathing. The endotracheal tube was disconnected from the ventilator during CA and no spontaneous breathing was observed. During this time anesthesia was not being delivered. Body warming was ceased 1 min prior to CA. During CA the pericranial temperature was maintained at 37.5 ± 0.5 °C by using a water-filled coil. Body temperature was allowed to fall spontaneously to 35 °C. Resuscitation was begun 8 min after the initiation of CA by slow injection of 0.2–0.5 mL of epinephrine (16 μg epinephrine/mL 0.9% saline), chest compressions at a rate of approximately 300 min^{-1} and resumption of ventilation with 100% FiO_2 at a rate of 210 breaths/min. Chest compressions were stopped upon ROSC, defined as electrical evidence of cardiac contractions. If ROSC was not achieved within 3 min of CPR initiation, resuscitation was stopped and the animal was excluded from the study. Five minutes following ROSC, FiO_2 was decreased to 50%. When the spontaneous respiratory rate was 30 breaths/min, the ventilator was adjusted to 150 breaths/min and when the animals had at least 60 spontaneous breaths/min, the endotracheal tube was removed. Temperature probes and intravascular catheters were removed and the surgical wounds were closed.

Following ROSC, animals in the normothermia group were rewarmed to reach 37 °C by using a heating lamp and pad at a rate of 0.3–0.5 °C per minute and maintained at 37 °C throughout the recovery. Animals in the hypothermia groups were allowed to have their body temperatures fall spontaneously to 32 °C or 30 °C and maintained for 30 min after CA/CPR when they were rewarmed to 37 °C as described above.

Mice were weighed and a health assessment score was calculated for each mouse daily for three days after CA/CPR by a blinded observer. The graded scoring systems ranged from 0 to 2, 0 to 3, or 0 to 5 depending on the behavior assessed, with 0 indicating no deficit and the upper limit indicating the most impaired. The behaviors assessed included consciousness (0–3), interaction (0–2), ability to grab wire top (0–2), motor function (0–5), and activity (0–2) (Allen et al., 2011; Deng et al., 2014). Scores in each category were summated to generate an overall health assessment score.

Hematoxylin & eosin staining

Three days after CA/CPR, animals were anesthetized with 3% isoflurane and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde. Brains were removed and post-fixed with paraformaldehyde and