**17β-ESTRADIOL PRETREATMENT REDUCES CA1 SECTOR CELL DEATH AND THE SPONTANEOUS HYPERTHERMIA THAT FOLLOWS FOREBRAIN ISCHEMIA IN THE GERBIL**

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Abstract—Pretreatment with 17β-estradiol attenuates ischemia-induced hippocampal cornu ammonis 1 (CA1) neuronal death. We assessed whether this is mediated through prevention of hyperthermia that normally follows ischemia in gerbils. Male gerbils were given sustained-released 17β-estradiol pellets or sham operation. Later, a guide cannula was implanted for brain temperature measurement and some were implanted with core temperature telemetry probes. Gerbils were subjected to either 5 min bilateral carotid artery occlusion or sham procedures 2 weeks after pellet surgery. Brain temperature was normothermic during surgery in all cases. In experiment 1, only core temperature was measured afterward in untreated and estrogen-treated gerbils. In experiment 2, postischemic core temperature was measured in untreated and two estrogen-treated ischemic groups, one of which had their postischemic temperature increased, via infrared lamp, to mimic the untreated group. Habituation was assessed on days 5 and 6. Hyperthermia, like that which occurs spontaneously, was forced on untreated and estrogen-treated ischemic animals in the third experiment, where brain temperature was measured. CA1 cell counts were assessed after a 7-day survival. A fourth experiment measured brain and core temperature simultaneously in normal gerbils during heating with an infrared lamp. Estrogen did not affect core temperature of non-ischemic gerbils whereas spontaneous postischemic hyperthermia was blocked. Estrogen reduced cell death and provided behavioral protection when gerbils regulated their own core temperature, but not when core hyperthermia was enforced. Conversely, estrogen reduced cell death in gerbils that had their brain temperature elevated. Experiment 4 showed that the brain becomes overheated (by approximately 1 °C) when core temperature is elevated. Accordingly, estrogen likely failed to reduce CA1 injury in experiment 2, when core hyperthermia was enforced, because of overheating the brain. In conclusion, estrogen reduces CA1 cell death by mechanisms other than preventing hyperthermia. Our results also suggest that future studies regulate brain instead of body temperature. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: stroke, estrogen, temperature, hippocampus, rodent, habituation.

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Abbreviations: CA1, cornu ammonis 1; E2, 17β-estradiol treatment; HOT, these groups were subjected to postischemic temperature regulation; ISC, ischemia; NT, no treatment; SHAM, surgery control procedure; $T_{brain}$, brain temperature; $T_{core}$, core temperature.

Clinical conditions of cerebral ischemia (ISC; e.g. stroke, cardiac arrest) are leading causes of death and disability. Many of the women who suffer a stroke may be taking some form of hormone replacement therapy, which is commonly used (Hersh et al., 2004). Compared with the controversial use of estrogen as a preventative agent against cardiovascular disease and the concerns with use of sex hormone replacement therapies such as increased breast cancer risk (Rossouw et al., 2002), there are many studies showing that estrogen is neuroprotective in female and male rodents (Roof and Hall, 2000; McCullough and Hurn, 2003). For example, estrogen reduces cell death in rodent models of global (Hurn et al., 1995; Jover et al., 2002; Shughrue and Merchenthaler, 2003) and focal ISC (Simpkins et al., 1997) as well as hemorrhagic stroke (Yang et al., 2001).

Hyperthermia and fever commonly accompany clinical conditions of ISC and are associated with worsened outcome (Hindfelt, 1976; Reith et al., 1996; Kammersgaard et al., 2002). Rodent studies confirm that hyperthermia worsens outcome. For example, induced hyperthermia aggravates injury after global ISC in rats even when heating is applied 24 h post-ISC (Baena et al., 1997). Rats and gerbils subjected to global ISC can also experience spontaneous postischemic hyperthermia (Colbourne and Corbett, 1994, 1995; Coimbra et al., 1996b). As well, the intraluminal suture occlusion model of stroke can cause delayed hyperthermia owing to hypothalamic damage (Zhao et al., 1994; Memezawa et al., 1995). Regardless of whether hyperthermia spontaneously occurs or results from a pathogen, it is clear that elevated brain temperature during or after ISC aggravates and potentially accelerates injury (Dietrich, 1992; Ginsberg and Busto, 1998). Furthermore, drugs that prevent hyperthermia/fever can be neuroprotective (Coimbra et al., 1996a,b) and induced hypothermia provides even greater benefit (Colbourne and Corbett, 1994, 1995). Accordingly, pharmacological neuroprotection studies (e.g. estrogen) should determine the drug’s effect on temperature because drug-induced cooling may be a confounding factor, as occurred with the putative neuroprotectant MK-801 (Buchan and Pulsinelli, 1990; Corbett et al., 1990). Indeed, clinical findings suggest that estrogen can lower temperature (Freedman and Blacker, 2002). Postmenopausal women taking estrogen replacement therapy tend to regulate their body temperature at a lower level than untreated women or those on estrogen and progestin therapy (Brooks et al., 1997).

In this study, we assessed whether 17β-estradiol reduces ischemic injury and behavioral abnormalities after forebrain ISC in gerbil, and whether the neuroprotection...
relates to changes in temperature. The gerbil bilateral common carotid artery occlusion model was chosen because: it consistently kills hippocampal cornu ammonis 1 (CA1) cells (Kirino, 1982; Colbourne and Corbett, 1994) as occurs following cardiac arrest in humans (Petito et al., 1987); the posts ischemic temperature profile is well established (Colbourne and Corbett, 1994, 1995); the model is commonly used to assess neuroprotection (DeBow et al., 2003); and previous studies show that estrogen reduces CA1 injury in this model and in male gerbils (Jover et al., 2003). Estrogen was administered before and after ISC as a sustained dose to mimic previous work (Jover et al., 2002).

**EXPERIMENTAL PROCEDURES**

**Animals**

All procedures in this study were in accordance with the Canadian Council on Animal Care guidelines as well as international standards, and were approved by a local animal care committee. Efforts were made to minimize animal suffering and to limit the number of animals required. In total, 102 adult male (approximately 75 g on the first surgery day) Mongolian gerbils were used. In experiment 1, these animals were obtained from Charles River (Montreal, QC, Canada) and in experiments 2, 3 and 4 these were obtained locally from stock originating from High Oak Ranch (Borden, ON, Canada). The gerbils were group-housed until the start of the experiment and then housed individually. Gerbils freely accessed food and water.

There were four groups in experiment 1: SHAM-17-estradiol treatment (E2; n=6 included), SHAM-no treatment (NT; n=7), ISC-NT (n=16), and ISC-E2 (n=18). Experiment 2 used three groups: ISC-E2 (n=11), ISC-NT (n=8) and ISC-E2-HOT (these groups were subjected to posts ischemic temperature regulation; n=9). Experiment 3 had two groups: ISC-NT-HOT (n=8) and ISC-E2-HOT (n=7). Experiment 4 used seven gerbils. Three had a cannula affixed to the skull using dental cement and four had only a burr hole with no dental cement. Seven additional gerbils were excluded due to problems unrelated to the effects of estrogen or ISC (e.g. telemetry probe failure).

**Estrogen pellet implantation**

Gerbils were s.c. implanted (dorsal neck) with either E2 pellets (0.36 mg pellet; 60 day controlled time release; Innovative Research of America Inc., Sarasota, FL, USA) or they were subjected to a sham operation and not treated (NT) with E2. The brief surgery was performed under isoflurane anesthesia (1.5%–2% maintenance in 70% N2O and 30% O2 mixture) using aseptic technique. The estrogen pellet size was based upon findings of Jover et al. (2002). Gerbils in experiment 4 did not undergo this surgery.

**Telemetry temperature probe surgery**

Eleven days following E2 pellet implantation/sham operation each gerbil was implanted (isoflurane anesthesia) with a core temperature (Tcore) telemetry probe (model TA10TA-F20; Transom Medical, St. Paul, MN, USA) into the abdominal cavity and a 5.0 mm guide cannula was cemented in place over the dura mater (approximately 1.0 mm rostral and 1 mm left of bregma) as previously described (DeBow and Colbourne, 2003). The cannula was used during the ISC/sham ISC procedure for the measurement of brain temperature (Tbrain) with an inserted thermocouple probe (model HYP 1-30-1/2-T-G-60-SMP-M; Omega Engineering, CT, USA) that measured the Tbrain of the dorsal striatum. Following surgery, animals were housed in a cage that rested on receivers (RPC-1; Transom Medical) that measured Tcore twice per minute via either Dataquest 3 or A.R.T. 2.2 software (Transom Medical). Gerbils in experiment 3 did not have a Tcore probe implanted. Instead, only the guide cannula was implanted for the subsequent use of a XM-FH Tbrain telemetry probe (Mini-Mitter Co., Bend, OR, USA) as previously described (DeBow and Colbourne, 2003). All gerbils in experiment 4 were implanted with a Tcore telemetry probe of which three animals were implanted with a guide cannula as done in experiment 3 and the remaining four received a burr hole without the use of a cannula and dental cement. The XM-FH and TA10TA-F20 telemetry probes were calibrated to the HYP 1-30-1/2-T-G-60-SMP-M thermocouple probe. See Table 1 for a list of groups and temperature measurement/control methods.

**Transient forebrain ISC**

Two weeks following the E2 pellet/sham surgery, gerbils were subjected to ISC or sham operation under isoflurane anesthesia. First, the Tbrain thermocouple probe (experiments 1 and 2) or Tbrain telemetry probe (experiment 3) was inserted through the guide cannula into the dorsal striatum. Tbrain was closely regulated at normothermic levels (approximately 36.3 °C) using an overhead infrared lamp (175W). ISC was produced by bilateral carotid artery occlusion (5 min) with micro-arterial clips. The sham procedure (SHAM) just involved carotid isolation. After clip removal, the

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**Table 1. Temperature measurement and regulation methods in experiments 1–4**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>ISC/SHAM groups</th>
<th>Intra-operative Tbrain regulation</th>
<th>Post-surgery measurement method</th>
<th>Post-surgery regulation</th>
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<td>Tcore regulated</td>
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* a See Fig. 1 for postoperative temperature data in experiments 1–3. Figs. 4 and 5 contain the temperature data from experiment 4. N/A, not applicable.
arteries were inspected to ensure reperfusion. Marcaine (Sanofi, Markham, ON, Canada) was applied to the wound and then it was sutured and a topical antibiotic ointment was applied. The $T_{\text{brain}}$ thermocouple probe was then removed and anesthesia stopped. In experiment 3, the $T_{\text{brain}}$ telemetry probe remained in place for 24 h before removal under brief intrasural anesthesia. Animals in experiment 4 were not subjected to ISC or the sham procedure. Instead, gerbils were anesthetized with isoflurane as described above, allowed to self-regulate their temperature for 15 min, then heated, via infrared lamp, to 39 °C for a total period of 30 min. When gerbils reached the target $T_{\text{core}}$ range, the lamp was turned off and they were allowed to spontaneously cool until 15 min following the 30 min heat period. During the entire procedure striatal $T_{\text{brain}}$ and telemetry $T_{\text{core}}$ were measured every minute. The methods for $T_{\text{brain}}$ and $T_{\text{core}}$ measurements were the same as described above. The thermocouple probe was held in place with a stereotaxic frame in the four gerbils where the probe was not secured through a guide cannula. At the end of this period, the experiment was finished. No histology or behavioral data were collected for animals in experiment 4.

**Post-ISC/sham operation temperature measurement/regulation**

$T_{\text{core}}$ was recorded following the ISC/SHAM procedure for 7 days in experiment 1, and 4 days in experiment 2. All core probes remained in place until killing. Postischemic $T_{\text{brain}}$ was measured for 24 h in experiment 3. Animals in experiment 1 controlled their own temperatures. Animals in experiment 2 either regulated their temperature (ISC-E2; ISC-NT) or were subjected to 8 h of temperature regulation (ISC-E2-HOT). The temperature of the latter group was forcibly increased via infrared lamps to achieve a profile similar to the ISC-NT group in experiments 1 and 2. Eight hours of temperature regulation was chosen, as the ISC-E2 and ISC-NT groups were similar after 8 h in experiment 1. Both ISC-NT-HOT and ISC-E2-HOT groups were heated in experiment 3 to mimic a $T_{\text{brain}}$ pattern that spontaneously occurs in this model (Colbourne and Corbett, 1994, 1995). Twenty-four hours of temperature regulation was used in experiment 3 to avoid rebound cooling.

**Habituation testing**

In experiment 2, gerbil activity was measured in a habituation test (Colbourne et al., 1998) for 15 min on days 5 and 6 post-ISC/SHAM operation. The test consisted of placing the gerbil in a small maze and measuring activity levels via the core telemetry probe. Activity counts (signal strength variations) were summed every 30 s and averaged every 5 min during the test. Activity levels correlate highly with CA1 injury (Colbourne et al., 1998). Animals with greater CA1 injury tend to be more active. Habituation activity was not assessed in the third experiment as the probes were removed at 24 h to avoid breakage.

**Assessment of hippocampal CA1 sector cell death**

Gerbils were killed 7 day after the ISC/SHAM procedure with an overdose of sodium pentobarbital (approximately 80 mg/kg i.p.) and perfused with 0.9% saline followed by 10% neutral buffered formalin. Ten micrometer coronal sections were obtained using a cryostat and stained with hematoxylin and eosin. Viable (not eosinophilic) neurons in the CA1 cell sector were counted and summed in the medial, middle, and lateral zones (each approximately 0.2 mm long) at −1.7 mm to bregma as described (Colbourne and Corbett, 1995). This coronal level correlates well with other levels (Colbourne and Corbett, 1995). Cell counts were expressed as a percentage of normal (i.e. average of SHAM-E2 and SHAM-NT groups in experiment 1), which did not affect the statistical analysis.

**Estradiol serum assay**

In experiments 2 and 3, cardiac blood samples were obtained 7 day post-ISC during euthanasia. The samples were refrigerated for approximately 2 h to allow for clotting. The blood samples were then centrifuged (13,000 RPM for 10 min) to obtain the serum. This was then stored at −20 °C and later analyzed via electrochemiluminescence immunoassay for the determination of E2 levels by our university hospital staff. Testosterone was also assayed but only in experiment 3.

**Statistics**

Data are presented as mean ± S.E.M. Activity data were analyzed with a two way repeated-measures (day and the 5 min blocks within the 15 min session per day) between subjects (group) ANOVA. Groups were compared via planned comparisons. CA1 cell counts, temperature and estrogen data were compared with two tailed t-tests that assumed or did not assume equality of variances depending upon a Levene’s test for homogeneity of variance. We did not correct for the significance level of 0.05 when multiple comparisons were made as we wished to avoid making type II errors (e.g. erroneously concluding that estrogen was not protective). Pearson’s correlations were also used.

**RESULTS**

**Experiment 1**

Baseline $T_{\text{core}}$ (24 h average on day prior to ISC/sham operation) ranged from 37.3 to 37.4 °C (Group effect: $P<0.651$). The first 8 h of $T_{\text{core}}$ following ISC/sham operation (Fig. 1A) were averaged and analyzed by ANOVA (Group effect: $P<0.001$). During this time the SHAM-E2 (37.2 ± 0.1) and SHAM-NT (37.2 ± 0.1) were not significantly different ($P=0.828$). Spontaneous hyperthermia occurred in the ISC-NT group (38.1 ± 0.1; $P<0.001$ versus combined SHAM group: 37.2 ± 0.1). The ISC-E2 group (37.3 ± 0.1) was not significantly hyperthermic (versus SHAM; $P=0.443$) and they were significantly ($P<0.001$) cooler than the ISC-NT group.

CA1 cell counts (Fig. 2A) were similar in SHAM-NT and SHAM-E2 groups ($P=0.454$), which were combined for subsequent comparisons. Cell death in the ISC-NT group was significantly attenuated in the ISC-E2 group ($P=0.041$).

**Experiment 2**

Baseline $T_{\text{core}}$ ranged from 37.2 to 37.5 (Group effect: $P=0.236$). An ANOVA was performed on the first 8 h of averaged postischemic temperature data revealing a significant group effect ($P<0.001$). As in the first experiment, the ISC-E2 group (37.0 ± 0.2) was significantly cooler during this time than the ISC-NT group (37.9 ± 0.3; $P=0.002$; Fig. 1B). The ISC-E2-HOT (38.1 ± 0.04) group was kept similar to the ISC-NT group ($P=0.606$) during the first 8 h and they were significantly warmer than ISC-E2 ($P<0.001$).

Cell counts significantly differed among groups ($P<0.001$). The ISC-E2 group had significantly more CA1 sector cells than the ISC-NT group ($P=0.004$; Fig. 2B). Enforced core hyperthermia (ISC-E2-HOT) blocked this neuroprotective effect ($P<0.001$ vs. ISC-E2; $P=0.853$ vs. ISC-NT).

An ANOVA on activity levels during the habituation tests (Fig. 3) revealed significant day and time main effects...
(P<0.001) as activity declined within a session and over days. The group main effect was also significant (P=0.003). The ISC-E2 group was significantly less active than the ISC-NT group (P=0.003). The ISC-E2-HOT group was similar to the ISC-NT group (P=0.821) and more active than the ISC-E2 group (P=0.003). Thus, estrogen attenuated the ISC-induced hyperactivity (i.e. habituation impairment), but not when temperature was controlled. Three gerbils were not tested due to an experimenter error.

Estrogen levels in the ISC-E2 (1845.3 pmol/L±520.2) and ISC-E2-HOT (2252.9±537.4) groups were not significantly different (P=0.596), but were significantly higher (P=0.013) than the ISC-NT group (129.4±13.4).

**Experiment 3**

The 8 h postischemic average of Tbrain was not significantly different (P=0.176) between ISC-NT-HOT (37.5±0.1) and ISC-E2-HOT groups (37.3±0.1; Fig. 1C). The ISC-E2-HOT group had significantly more CA1 cells (P=0.046; Fig. 2C).

![Fig. 1. Tcore (°C) following ISC/sham operation in experiments 1–3 (A–C, respectively). Data are shown for the first 48 h in experiments 1 and 2 as subsequent temperatures were quite similar. Only 24 h of data were collected in the last study. Temperature was not regulated in experiment 1 whereas the ISC-E2-HOT group was regulated in experiment 2. Both groups were regulated in the third experiment (see Experimental Procedures).](image)

![Fig. 2. CA1 sector cell counts (% normal) at -1.7 mm to Bregma for experiments 1–3 (A–C, respectively). * Denotes a significant difference from the ischemic group that did not receive estrogen.](image)
terone \((r=-0.687, \ P=0.005)\). Estrogen \((r=0.685, \ P=0.005)\), but not testosterone \((r=-429, \ P=0.111)\), significantly predicted CA1 counts.

**Experiment 4**

Temperature profiles of those with and without a cannula assembly were quite similar and thus these data were pooled (Fig. 4). The average \(T_{\text{brain}}\) (35.8 ± 0.4) and \(T_{\text{core}}\) (35.8 ± 0.3) were not different during the 15 min period prior to heating (i.e. baseline condition). Likewise, \(T_{\text{brain}}\) (37.7 ± 0.6) and \(T_{\text{core}}\) (38.0 ± 0.6) did not significantly differ in the period following heating \((P=0.360)\). During heating, \(T_{\text{brain}}\) (38.1 ± 0.4) was on average 1 °C higher than \(T_{\text{core}}\) (37.1 ± 0.1). Indeed, the difference between \(T_{\text{brain}}\) and \(T_{\text{core}}\) (i.e. \(T_{\text{brain}}-T_{\text{core}}\)) during the baseline versus heating condition was significant \((P<0.001)\). Thus, the \(T_{\text{brain}}\) rose proportionately more during the heating period than \(T_{\text{core}}\) did.

Significant correlations were found between \(T_{\text{brain}}\) and \(T_{\text{core}}\) during baseline \((r=0.895, \ P<0.001)\) and heating conditions \((r=0.873, \ P<0.001)\). Furthermore, the relationship between \(T_{\text{brain}}-T_{\text{core}}\) (i.e. magnitude of overheating) and \(T_{\text{core}}\) was weak during baseline (Fig. 5a; \(r=0.275, \ P=0.004\)) and not significant during heating (Fig. 5b; \(r=-0.080, \ P=0.248\)). Thus, at least under anesthesia (no ISC), \(T_{\text{core}}\) predicts \(T_{\text{brain}}\) well. Furthermore, regulation of body temperature (i.e. heating condition) results in a relatively consistent overheating of the brain, at least in this paradigm (e.g. under anesthesia, \(T_{\text{core}}\) range of 35–39 °C).

**CONCLUSIONS**

The use of 17β-estradiol, starting 2 weeks prior to ISC, significantly reduced CA1 cell death and concomitant functional abnormalities, as previously found (Hurn et al., 1995; Sudo et al., 1997; Jover et al., 2002; McCullough and Hurn, 2003; Shughrue and Merchenthaler, 2003). Importantly, estrogen blocks the spontaneous postischemic \(T_{\text{core}}\) hyperthermia. Our second experiment suggests that estrogen’s neuroprotective effect is solely due to preventing spontaneous hyperthermia because maintenance of \(T_{\text{core}}\) hyperthermia to control levels completely abolished the protective effect of estrogen. Surprisingly, a third experiment that controlled \(T_{\text{brain}}\) after ISC suggests that estrogen significantly reduces CA1 cell death by additional mechanisms as enforcing \(T_{\text{brain}}\) hyperthermia did not abolish the neuroprotective effect of estrogen.

There are several possible explanations for the attenuated postischemic hyperthermia found with estrogen (experiments 1 and 2). First, estrogen may act like an antipyretic agent. Indeed, estrogen can affect thermoregulation (Tataryn et al., 1980; Freedman and Blacker, 2002). We did not assess whether prevention of fever alone would reduce cell death because forced cooling is stressful (MacLellan et al., 2004) and antipyretic drugs often have additional effects (e.g. anti-inflammatory). Second, estrogen may attenuate the ischemic insult resulting in a diminished level of postischemic hyperthermia. Notably, postischemic hyperthermia varies with ISC duration (Colbourne and Corbett, 1994). Finally, both possibilities may occur concomitantly. Given the significant protection found in experiment 3, in which postischemic \(T_{\text{brain}}\) was regulated, most of estrogen’s neuroprotective ef-

![Fig. 3.](image-url) Activity counts during habituation maze testing on days 5 and 6 post-ISC in experiment 2. Activity was measured by the telemetry system as movement of the probe across the receiver (signal strength changes). The 30 s summed counts were averaged every 5 min of the 15 min test sessions. Higher activity counts are indicative of habituation impairment and correlate highly with CA1 sector damage (Colbourne et al., 1998). See Results for statistics.

![Fig. 4.](image-url) Averaged \(T_{\text{core}}\) and \(T_{\text{brain}}\) of gerbils in experiment 4 during heating schedule consisting of 15 min of baseline, 30 min of heating (until \(T_{\text{core}}\) of 39 °C was reached) and 15 min of spontaneous cooling.
effect cannot be explained by an antipyretic mechanism. However, an effect of estrogen on thermoregulation may better explain the spontaneous and long-lasting mild hypothermia that occurred following temperature regulation in the ISC-E2-HOT group in experiment 2, which is probably also due to the increased efficacy of cooling mechanisms (e.g. peripheral vessel dilation, licking of fur) when the external heating stopped.

Unexpectedly, experiments 2 and 3 led to different conclusions. Accordingly, we hypothesized that elevating $T_{\text{core}}$ via heating lamps disrupts the normally good relationship between $T_{\text{brain}}$ and $T_{\text{core}}$ during baseline (A) and heating conditions (B). A close relationship (i.e. $T_{\text{brain}} - T_{\text{core}}$ values near zero) was found during baseline readings. However, during the period of elevating $T_{\text{core}}$, we found that $T_{\text{brain}}$ was approximately 1 °C warmer. All data points for each animal are shown (e.g. 15 data points, one per minute, per animal during baseline).

Fig. 5. The relationship (line denotes best fitting linear regression) between $T_{\text{brain}} - T_{\text{core}}$ and $T_{\text{core}}$ during baseline (A) and heating conditions (B). A close relationship (i.e. $T_{\text{brain}} - T_{\text{core}}$ values near zero) was found during baseline readings. However, during the period of elevating $T_{\text{core}}$, we found that $T_{\text{brain}}$ was approximately 1 °C warmer. All data points for each animal are shown (e.g. 15 data points, one per minute, per animal during baseline).

is required to determine if the magnitude of brain overheating is similar at other temperature ranges, with alternative heating methods (e.g. heating pad), in rats and mice, and in non-anesthetized animals. Unfortunately, it is difficult to test whether similar differences exist in non-anesthetized rodents. First, measuring $T_{\text{core}}$ and $T_{\text{brain}}$ simultaneously using telemetry is difficult because the two signals interfere with one another. Second, wired thermocouple systems risk causing stress which may confound the study (e.g. by causing stress-induced fever). Third, the use of a large head cap assembly, such as that required to secure a brain probe in rats, may directly interfere with temperature (e.g. enhancing heat dissipation). Indeed, this likely explains why $T_{\text{brain}}$ is approximately 1 °C lower than $T_{\text{core}}$ when both are measured with telemetry probes (DeBow and Colbourne, 2003).

There are several limitations with our study. First, the supraphysiological levels of 17$\beta$-estradiol obtained are greater than those of Jover et al. (2002). Notably, we used the same pellet size supplied by the same manufacturer and obtained a comparable level of protection. Thus, the difference between these studies is likely due to the differing techniques of estradiol immunoassay analysis (Stanczyk et al., 2003). Second, high levels of estrogen lowered testosterone levels. As estrogen, not testosterone, significantly correlated with CA1 survival, it appears that estrogen is the critical hormone for neuroprotection. Third, we did not compare males and females, but our results suggest that estrogen would also benefit females. However, comparing across several of our studies it appears that there are no differences in the amount of CA1 injury sustained in males and females subjected to a 5 min insult, nor are there notable differences in the level of spontaneous hyperthermia. It is likely, therefore, that high levels of estrogen are required to protect against this severe insult and prevent hyperthermia. Fourth, while our results strongly suggest that estrogen mitigates the ischemic insult, thereby limiting postischemic hyperthermia, this study did not determine how this mitigation occurs (e.g. improving cerebral blood flow; He et al., 2002). Our study simply focused on postischemic temperature alterations. Fifth, further experiments should investigate the influence of postischemic administration of estrogen, not only to determine efficacy (for acute stroke intervention), but its effects on temperature as well need to be characterized.

In summary, there are three main findings in this study. First, estrogen significantly reduces CA1 cell death as shown in three separate experiments. Second, estrogen blocks spontaneous postischemic hyperthermia, which is not essential for neuroprotection. Nonetheless, in many cases fever aggravates injury (Ginsberg and Busto, 1998) and attenuating fever might provide benefit under other circumstances. Accordingly, estrogen should be re-evaluated in the middle cerebral artery occlusion (Zhao et al., 1994; Memezawa et al., 1995) and two-vessel occlusion models in rat (Coimbra et al., 1996a,b) where fever occurs and contributes to damage. Third, and equally important, the regulation of body temperature following ISC will not necessarily yield similar data to studies in which $T_{\text{brain}}$ is regulated. This is likely due to a disproportionate overheating of the brain with maintenance of $T_{\text{core}}$; a phenomenon which warrants further study.
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REFERENCES


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