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Translating Drug-Induced Hibernation to Therapeutic Hypothermia

Tulasi R. Jinka[†,‡], **Velva M. Combs**[†], and **Kelly L. Drew**[†,*]

[†]University of Alaska Fairbanks, 902 North Koyukuk Drive, Fairbanks, Alaska 99775-7000, United States

[‡]University of Michigan, Ann Arbor, Michigan 48109, United States

Abstract

Therapeutic hypothermia (TH) improves prognosis after cardiac arrest; however, thermoregulatory responses such as shivering complicate cooling. Hibernators exhibit a profound and safe reversible hypothermia without any cardiovascular side effects by lowering the shivering threshold at low ambient temperatures (T_a). Activation of adenosine A_1 receptors (A_1ARs) in the central nervous system (CNS) induces hibernation in hibernating species and a hibernation-like state in rats, principally by attenuating thermogenesis. Thus, we tested the hypothesis that targeted activation of the central A_1AR combined with a lower T_a would provide a means of managing core body temperature (T_b) below 37 °C for therapeutic purposes. We targeted the A_1AR within the CNS by combining systemic delivery of the A_1AR agonist ^{6}*N*-cyclohexyladenosine (CHA) with 8-(*p*-sulfophenyl) theophylline (8-SPT), a nonspecific adenosine receptor antagonist that does not readily cross the blood–brain barrier. Results show that CHA (1 mg/kg) and 8-SPT (25 mg/kg), administered intraperitoneally every 4 h for 20 h at a T_a of 16 °C, induce and maintain the T_b between 29 and 31 °C for 24 h in both naïve rats and rats subjected to asphyxial cardiac arrest for 8 min. Faster and more stable hypothermia was achieved by continuous infusion of CHA delivered subcutaneously via minipumps. Animals subjected to cardiac arrest and cooled by CHA survived better and showed less neuronal cell death than normothermic control animals. Central A_1AR activation in combination with a thermal gradient shows promise as a novel and effective pharmacological adjunct for inducing safe and reversible targeted temperature management.

Graphical abstract

[*]**Corresponding Author**. Address: 902 N. Koyukuk Dr., University of Alaska, Fairbanks, AK 99775-7000. kdrew@alaska.edu. Phone: (907) 474-7190. Fax: (907) 474-7666.

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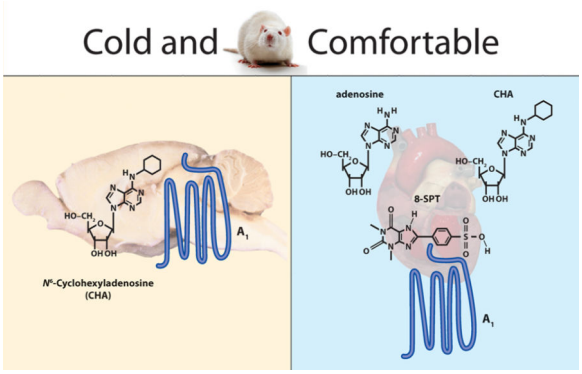

Keywords

Adenosine; hibernation; cardiac arrest; targeted temperature management; A_1AR and global cerebral ischemia; CHA; 8-SPT

INTRODUCTION

Mild therapeutic hypothermia, in which the core body temperature (T_b) is reduced to 32–34 °C for ≥24 h, is becoming the standard of care for cardiac arrest patients.[1] However, technical challenges may limit the use of therapeutic hypothermia. Shivering is one of the most problematic issues in targeted temperature management (TTM) and is controlled with pharmacological adjuncts, such as paralytics, narcotics, sedatives, or a combination of these such as meperidine and buspirone.[2,3]

Here we study ^{6}*N*-cyclohexyladenosine (CHA), an A_1 adenosine receptor (A_1AR) agonist found to induce hibernation,[4] as a novel pharmacological adjunct, to facilitate effective techniques for TTM. T_b in hibernating ground squirrels can fall to as low as −3 °C,[5] through a process regulated by A_1AR signaling within the central nervous system (CNS),[4,6] a mechanism common to other types of torpor.[7,8] Activation of the CNS A_1AR suppresses shivering and nonshivering thermogenesis. [9–12] A_1AR agonists protect against ischemic injury and seizure but have not been used clinically because of side effects, principally hypothermia, bradycardia, and hypotension.[13] Given the central site of action for A_1AR-mediated inhibition of thermogenesis, this study tests the hypotheses that (i) A_1AR agonist-induced cooling can be modulated by ambient temperature (T_a), (ii) A_1AR-mediated bradycardia can be managed by co-administration of an adenosine receptor antagonist that does not penetrate the blood–brain barrier, and (iii) reversal of bradycardia will occur without interfering with the cooling effects of CHA. Finally, we use a rat model of cardiac arrest to provide a proof of concept that this approach to TTM will improve survival and decrease the extent of brain injury following cardiac arrest.

RESULTS AND DISCUSSION

CHA Induces a Decrease in T_b and Heart Rate

Overcoming thermoregulation is one of the most problematic issues in targeted temperature management (TTM). Shivering is controlled with pharmacological adjuncts, such as paralytics, narcotics, sedatives, or a combination of these such as meperidine and buspirone,[2,3] with little regard to mechanism of action. Here we tested the concept that hypothermia in rats could be induced by mimicking the central A_1AR mechanism used by hibernating species to enter hibernation. Drugs were administered systemically because this is the most feasible route of administration in a clinical setting. We report that CHA [1.0 mg/kg, intraperitoneally (IP)] decreased T_b to 33 °C within 1 h (Figure 1A). Data show that repeated injection of CHA and 8-SPT at 4 h intervals (which approximates the half-life of CHA) induces a steady minimum in T_b after the fourth injection (Figure 1A). When cold, animals show neurological deficits consistent with low T_b values (Figure 1B). All animals were rewarmed after the drug was discontinued and they were moved to a T_a of 20 °C. No adverse events were noted after rewarming from an assessment of neurological deficit (Figure 1B). At 24 h, there was evidence of a slight but statistically significant bradycardia in CHA-treated rats (Figure 1C). The heart rate remained significantly elevated 5.5 h after the last CHA and 8-SPT injections, consistent with enhanced thermogenesis following cessation of CHA administration (Figure 1C) [$p < 0.0001$; two-way analysis of variance (ANOVA); time × treatment; $n = 6$].

The CHA-Induced Decrease in T_b Depends on T_a

If CHA-induced cooling is due to inhibition of thermogenesis, we predicted that the magnitude of cooling should depend on the thermal gradient, i.e., the difference between T_b and T_a, as seen during hibernation. To address this question, we employed programmable minipumps to deliver CHA continuously for 24 h to rats housed at a T_a of 16 or 25 °C. Results show that continuous administration of CHA at T_a values of 16 and 25 °C decreases T_b ($p < 0.0001$) and heart rate {$p < 0.01$; three-way ANOVAs, main effects of group [CD vs CHA (Figures 2 and 3)]} with no effect on hemoglobin oxygen saturation (sO_2) (not shown). Moreover, T_b was lowest at a T_a of 16 °C. At a T_a of 16 °C, the mean minimum T_b was 29.3 ± 0.3 °C (Figure 2), and at a T_a of 25 °C, the mean minimum T_b was 35.6 ± 0.1 °C. CHA is thought to facilitate the onset of torpor by suppressing thermogenesis via activation of the A_1AR within the CNS.[11,14] Core heat then dissipates at rates governed by T_a and thermal conductance. These results are consistent with this mechanism of action because the magnitude of CHA-induced cooling increased with a decrease in T_a. Although systemic administration is more likely to translate to a clinical scenario than ICV administration, direct stimulation of the A_1AR on the heart produces profound bradycardia.[11,15]

A_1AR Antagonist 8-SPT Reverses Bradycardia during Therapeutic Hypothermia without Affecting T_b

Knowing that CHA induces torpor via effects on the brain suggested that an A_1AR antagonist with poor blood–brain barrier permeability might reverse bradycardia while sparing the decrease in T_b. CHA lowered T_b when 8-SPT was administered 15 min prior to CHA in the preceding experiment. However, the short half-life of 8-SPT (45 min in rabbit[16])

suggested that heart rate measured 4 h after the last 8-SPT injection under-reported the full effect of 8-SPT because the drug would have been cleared by this time. Thus, during the 24 h period of continuous CHA administration, 8-SPT (or saline vehicle) was delivered, and heart rate and sO_2 were monitored at 10 min intervals for 60 min. At a T_a of 16 °C, in control (CD-treated) animals, both 8-SPT and saline vehicle increased heart rate. The increase in HR in both of these groups was interpreted as an effect of the injection because the effect of 8-SPT did not differ from the effect of saline [$p = 0.0434$; two-way ANOVA; main effect of time (Figure 2A,B)]. By contrast, in animals treated with CHA, 8-SPT (and not the vehicle saline) produced a 2-fold increase in heart rate ($p < 0.0001$; two-way ANOVA; time × treatment) with no influence on T_b (Figure 2C,D). As expected because of the short half-life of 8-SPT, the effects on heart rate subsided within 60 min of drug administration. Bradycardia at −1 h is not apparent in Figure 2C most likely because of random error in heart rate measurement. Bradycardia is evident in Figure 1C after the subjects had been cooled for 24 h and in Figure 2D during continuous CHA administration.

At a T_a of 25 °C, 8-SPT and saline vehicle delivered to control (CD treated) animals had no effect on heart rate or T_b (Figure 3A,B). However, when 8-SPT was delivered to CHA-treated animals, this adenosine receptor antagonist produced a small but significant increase in heart rate ($p < 0.0001$; two-way ANOVA; time × treatment) with no influence on T_b (Figure 3C,D). Under these conditions, CHA induced only a slight decrease in T_b and no significant decrease in HR, showing that cooling is more effective at producing bradycardia than this dose of CHA. Importantly, the magnitude of the effect of 8-SPT on heart rate was greater in animals housed at a T_a of 16 °C than on those housed at a T_a of 25 °C (Figures 2 and 3). These results show that both hypothermia and CHA contribute to an adenosine receptor-mediated bradycardia, both of which can be managed with 8-SPT without compromising hypothermia.

The time to target temperature may influence outcome. Thus, control over time to target T_b is desired even though optimal timing remains an area of active research.[17] Here we show that pharmacological intervention with adjustments in the temperature differential can be used to manage the rate of cooling and maintenance of hypothermia; animals were cooled faster and to a lower T_b at the colder T_a. In addition, continuous administration of CHA produced a faster decline and a steadier minimum T_b when compared to intermittent injections. A_1AR agonists applied too soon after the ischemic event, however, could be detrimental. An increased level of adenosine signaling immediately after traumatic brain injury contributes to respiratory depression and death such that caffeine, a nonselective adenosine receptor antagonist, prevents acute mortality when administered immediately after traumatic brain injury.[18] Other studies show that a longer time lag between the return of spontaneous circulation (ROSC) and target T_b is associated with a more favorable neurologic outcome in patients after cardiac arrest when compared to patients with a shorter time lag between ROSC and target T_b.[17] Nonetheless, because the rate of CHA-induced cooling and minimum T_b depend on the temperature differential, one is able to adjust the timing and depth of hypothermia through control of the temperature differential, i.e., the heat sink. The temperature differential may be achieved through surface cooling, intravascular cooling,[19,20] or T_a. Moreover, the ability to control the rate and depth of

cooling with T_a is consistent with other evidence that CHA produces a decrease in T_b by inhibiting thermogenesis.[9]

CHA-Induced Therapeutic Hypothermia following Cardiac Arrest Decreases the Extent of Brain Damage

Hypothermia is well-known to enhance survival after cardiac arrest.[21] To ensure that CHA or 8-SPT did not interfere with the documented benefits of TH, we next sought to test if CHA-induced TH in conscious rats would improve survival and decrease the extent of brain damage following global cerebral ischemia using a model of asphyxial cardiac arrest (CA). With this model, in our hands, asphyxia for 6 and 8 min produces a similar loss of CA1 neurons 8 days after ROSC.[16]

Rats subjected to CA for 8 min and treated with 8-SPT and CHA at a T_a of 16 °C survived better than the normothermic control (NC) group. At the onset of treatment, T_b was 33.5 ± 0.1 and 33.5 ± 0.1 °C in the NC and TH groups, respectively. T_b in all three NC rats increased to 36.5–36.8 °C within 15 min of placement at 29 °C and remained between 36.2 and 37.3 °C until death. Only one rat in the NC group survived to 8 days. The two remaining rats died between 13 and 18 h after ROSC (Figure 4). T_b in the three rats treated with TH decreased to 31.0–31.6 °C within 3 h of CHA injection and remained between 31.8 and 29.2 °C for 24 h before the rats were rewarmed; the mean of individual T_b minima was 29.7 ± 0.3 °C. Rats were rewarmed without intervention within 5 h of transfer to a T_a of 20 °C. Transfer occurred 4 h after the last injection of CHA. All three rats treated with TH survived for 8 days despite a CA-induced decrease in MABP similar to that of the NC group (Figure 4). Histopathology showed ischemia-induced cell death of CA1 neurons in the hippocampus of the one normothermic control animal that survived for 8 days (Figure 5). The number of healthy neurons per millimeter of CA1 (mean ± standard error of the mean) was 135.5 ± 4.6 for the TH group ($n = 3$) and 47.8 for the normothermic control group ($n = 1$). This compares to the value of 180.8 ± 12.3 for naïve rats ($n = 5$) reported previously.[16] The hypothermic group showed better survival but was also exposed to a lower T_a. A lowered T_a is unlikely to have improved survival unless it also affected core T_b. This is because, without CHA, a lowered T_a stimulates thermogenesis, and the greater metabolic demand would be expected to worsen the outcome.

In addition to effects on thermogenesis, A_1AR agonists are neuroprotective in animal models of cardiac arrest[22] and attenuate seizure activity.[23] These direct effects within the CNS may have contributed to enhanced survival in this study and contribute to the therapeutic efficacy of these drugs if used as pharmacological adjuncts for TH. Importantly in this study, enhanced survival was observed relative to a NC group where T_b varied between 33.5 and 37.1 °C. In no case did T_b exceed 37.1 °C in the NC group at any of the times measured. Thus, enhanced survival is not due to the absence of hyperthermia, an issue emphasized in a recent clinical study that questions the value of cooling.[24]

These results show that A_1AR agonist CHA, combined with a decrease in T_a, is an effective adjunct for inducing TH. Decreasing T_a to 16 °C and administering CHA intermittently or continuously produces a rapid and sustained decrease in core T_b with minimal effects on heart rate. Moreover, slight bradycardia is readily reversed with 8-SPT, which has no effect

on T_b. We, and others, have shown that activation of CNS adenosine A_1 receptors (A_1ARs) is necessary and sufficient to induce hibernation and torpor in mice.[4,7,8] These results suggest that some of the torpor-inducing effects of CHA may translate to refined methods of facilitating hypothermia for therapeutic purposes. Pharmacological management of shivering and nonshivering thermogenesis is necessary to counteract normal thermoregulatory mechanisms, especially shivering.[2] Cold infusions alone do not keep patients cool.[25] In comatose cardiac arrest patients, shivering may be controlled by a number of pharmacological adjuncts, including sedatives, narcotics, and paralytics.[2] Surface cooling can cause skin lesions when shivering is not adequately controlled.[26] More importantly, shivering can prevent the attainment of the target temperature and contribute to adverse effects of TH. Shivering is especially difficult to manage in conscious patients,[3] which may limit the benefit of TH in stroke patients.[27] A recent study in rats[9] shows that CHA acts, in part, within the nucleus of the solitary tract (NTS) to produce effects that closely resemble the suite of thermoregulatory and autonomic nervous system changes that accompany the onset of hibernation.[14,28,29] These data suggest that A_1AR agonists are promising pharmacological adjuncts for TH for the treatment of stroke, cardiac arrest, and other conditions.[30] The data shown here with CHA complement prior work using 5′AMP as an A_1AR agonist to induce TH, an approach found to reduce infarct size following middle cerebral artery occlusion in rats.[12]

METHODS

Sustained Hypothermia in Conscious Rats

Experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals, 8th Edition (National Research Council, National Academies Press, 2010), and protocols were approved by the Institutional Animal Care and Use Committee of the University of Alaska Fairbanks. Male Sprague-Dawley rats (2–3 months old, 375–400 g; obtained directly or derived from breeders obtained from Simonson Laboratories, Gilroy, CA) were housed in pairs at 20 °C on a 12L:12D photoperiod, fed ad libitum, and allowed at least 2 weeks to acclimate before being used.

Implantation of Data Loggers and a Programmable Pump

Baytril [5 mg/kg, subcutaneously (SC)] began 24 h before surgery and continued BID for 3 days. On the day of surgery, isoflurane anesthesia mixed with medical grade O_2, delivered at a rate of 1.5 L/min, was induced at 5% and maintained at ≤2% depending on respiratory frequency. Surgery was followed by 10–14 days of postoperative recovery. iButton data loggers (Maxim Integrated, San Jose, CA) were implanted IP alone or in combination with an iPRECIO pump (Data Sciences International, St. Paul, MN). Pumps were implanted SC at the back of the neck. The outlet tube on the pump was tunneled subcutaneously from the dorsal pocket to the incision site on the neck and secured. Prior to implantation, the pumps were programmed to deliver at a rate of 1.0 μL/h except on the two experimental days, during which the flow rate increased to 30.0 μL/h for 24 h. CHA or vehicle delivery commenced just after residual saline was withdrawn and the 900 μL reservoir filled with CHA or vehicle. Delivery ended after 24 h when residual CHA or vehicle was withdrawn and the reservoir refilled with saline.

Drugs

N^6-Cyclohexyladenosine (CHA) and 8-(*p*-sulfophenyl)-theophylline hydrate (8-SPT) were purchased from Sigma-Aldrich (St. Louis, MO). Hydroxypropyl-β-cyclodextrin (CD) was purchased from TCI America (Portland, OR).

Drug Delivery via Sequential IP Injections

Animals were instrumented with IP iButton data loggers (Maxim Integrated) programmed to record body temperature (T_b) every 10 min and allowed 14 days postoperative recovery prior to drug testing. ^{6}*N*-Cyclohexyladenosine (CHA) (A_1AR agonist) was dissolved in 0.01 M phosphate buffer (PB); 8-(*p*-sulfophenyl)theophylline (8-SPT) (non-selective adenosine receptor antagonist) was dissolved in 0.9% saline and filter sterilized on the day of administration. PB for CHA and saline for 8-SPT were administered as vehicle controls where indicated. The day before the experiment, animals in both treatment and control groups were moved to a T_a of 16 °C and remained at this T_a until they were returned to a T_a of 20 °C, 4 h after the last injection. Animals in the treatment group received a total of six injections of CHA (1.0 mg/kg, IP) every 4 h and a total of six injections of 8-SPT (25 mg/kg, IP), administered 15 min prior to each CHA injection. The control group received the same number of injections, with 8-SPT replaced by saline and CHA replaced by PB. Treatment and control conditions were tested in all animals with at least 1 week between experiments using a balanced crossover design such that one-half of the animals received CHA and 8-SPT during the first experiment and the other half received CHA and 8-SPT during the second experiment. Except for moving rats to a T_a of 20 °C 4 h after the last injection, no other means were used to facilitate rewarming. Neurological deficits, heart rate, and sO_2 were measured 2 h and immediately before injection, at 24 h, after the rats had been rewarmed and daily for the next 3 days using a pulse oximeter applied to the hind paw (Vet/Ox TM 4402L, Sensor Devices, Waukesha, WI). The total neurological deficit score (NDS) consists of five components: consciousness and respiration, cranial nerve function, motor function, sensory function and coordination (leg/tail movement, cleaning, depth perception, and righting reflex), and motor and sensory function as described previously.[31] NDS ranges between 0 (no neurological deficiency, normal function) and 100 (maximal neurological deficiency). Healthy neurons in CA1 were counted as described previously.[16]

Drug Delivery through iPRECIO Pumps

To determine if the effects of CHA on heart rate were due to direct effects of CHA on the heart or the effects of tissue temperature, we employed programmable peristaltic minipumps to deliver CHA continuously for 24 h at T_a values of 25 and 16 °C. During constant delivery of agonist, we also tested the effects of 8-SPT on heart rate and hemoglobin saturation (sO_2) at 10 min intervals appropriate for the short half-life of the drug. For these experiments, rats were instrumented with programmable iPRECIO pumps (Data Sciences International). CHA was dissolved in 25% (w/v) hydroxypropyl-β-cyclodextrin (CD) in sterile water, and 8-SPT was dissolved in 0.9% saline. Pumps delivered the same mass of CHA as in the first experiment (6 mg/kg over a 24 h period); however, in this case, CHA was delivered at a constant rate of 30 μL/h. HR and sO_2 were monitored using a pulse oximeter every 10 min for 1 h following a single injection of 8-SPT (25 mg/kg, IP) or vehicle as indicated. As

before, animals in both treatment and control groups were moved to a T_a of 16 or 25 °C the day before the experiment and remained at this T_a until they were returned to a T_a of 20 °C, 4 h after the end of drug delivery. Drug and vehicle treatments were administered to all animals using a balanced crossover design. At least 1 week separated CHA and vehicle (CD) treatment, and 1 h separated 8-SPT and vehicle (saline) injections.

Therapeutic Benefit of Sustained Hypothermia in Conscious Rats Subjected to Asphyxial Cardiac Arrest

Rats 68–75 days of age were subjected to asphyxial cardiac arrest for 8 min, and animals that were resuscitated within 120 s and met additional inclusion criteria 60 min after ROSC (Table 1) were randomly allocated to a therapeutic hypothermia (TH) or a normothermic control (NC) group using a computer-generated randomization schedule (http://www.jerrydallal.com). Treatment commenced 70 min after ROSC. Animals assigned to the TH group were moved to 16 °C and CHA and 8-SPT delivered as described above for Drug Delivery via Sequential IP Injections. Animals assigned to the NC group were moved to a neonatal incubator set to 29 °C and vehicles (PB and saline) delivered as described above for the control group (see Drug Delivery via Sequential IP Injections). At the end of 24 h, all rats were moved to and housed at an T_a of 20 °C for 7 days until they were euthanized for tissue collection. Body temperature was monitored prior to each injection throughout the treatment and daily thereafter using SC IPTT-300 transponders (BioMedic Data Systems, Inc., Seaford, DE).

Statistics

Data are reported as means ± the standard error of the mean unless otherwise indicated. Data were analyzed by two-way ANOVA with repeated measures over time and Tukey post hoc comparisons (SAS, version 9.1.3) or a *t* test (Excel 2010) where indicated.

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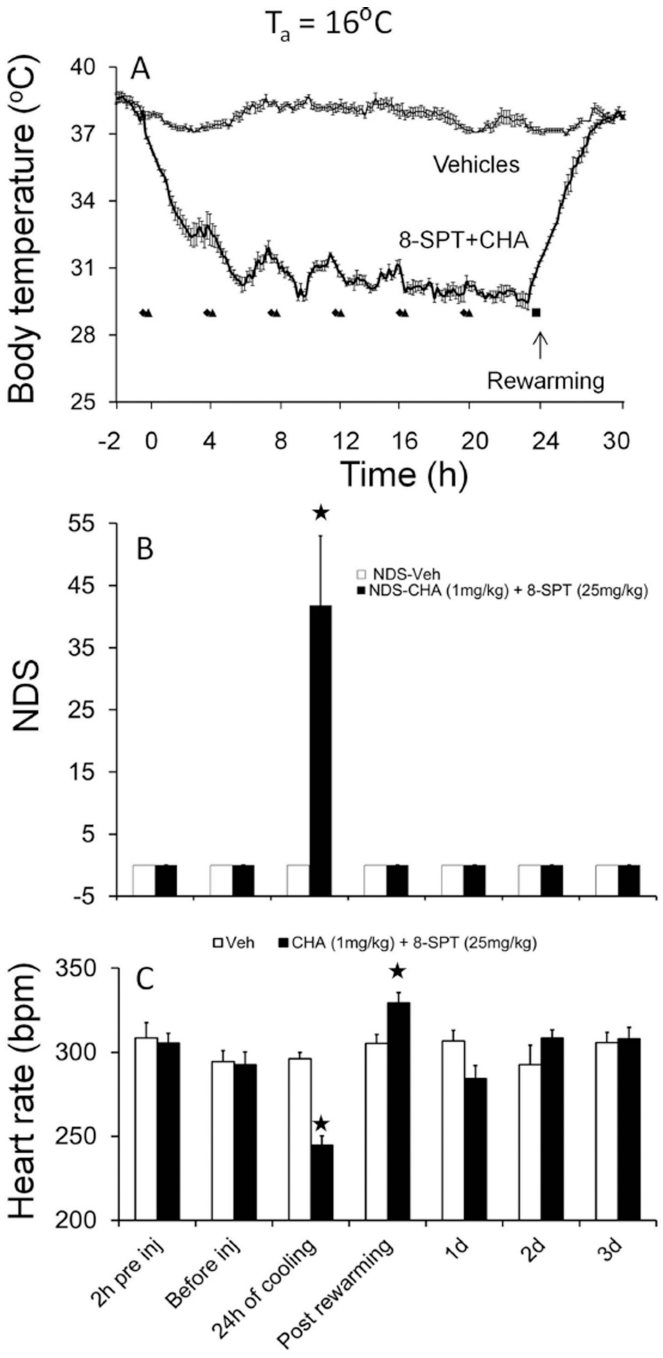

Figure 1.

(A) CHA at a T_a of 16 °C induces hypothermia and heart rate changes. CHA injected every 4 h induces and maintains moderate hypothermia for 24 h. 8-SPT was administered 15 min prior to CHA: (◆) time of 8-SPT injection and (▲) time of CHA injection. Vehicle injections [saline and phosphate buffer (PB)] had no effect. (B) Neurological deficit scores (NDSs) increase during hypothermia. (C) Heart rate decreases during cooling and increases after rewarming. Data shown are means ± the standard error of the mean. ★$p < 0.05$ vs veh [saline and phosphate buffer (PB)] (Tukey test; $n = 6$ per group).

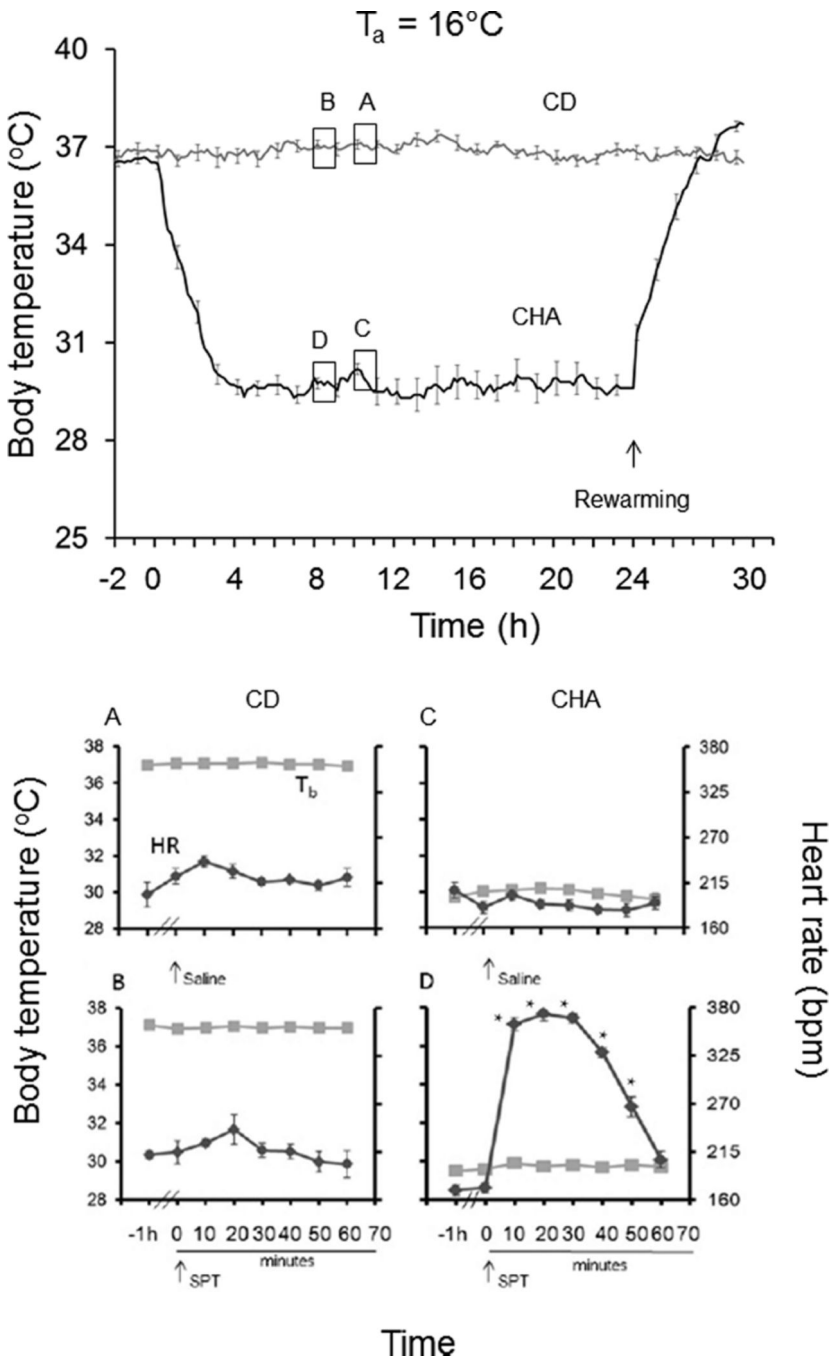

Figure 2.

Continuous CHA delivery (30 μL/h) via a SQ minipump at a dose equivalent to 1.0 mg/kg every 4 h at a T_a of 16 °C produces stable hypothermia (top). 8-SPT (25 mg/kg, IP) delivered 8 h after the onset of CHA delivery (B and D) increases heart rate without affecting body temperature. 8-SPT vehicle (saline) delivered 1 h later had no effect (A and C) (★$p < 0.05$ vs an analogous time point shown in panel C; Tukey test; $n = 5$ per group). The symbol and line style defined as T_b in panel A denotes T_b in panels B–D. The symbol and line style defined as HR in panel A denotes HR in panels B–D.

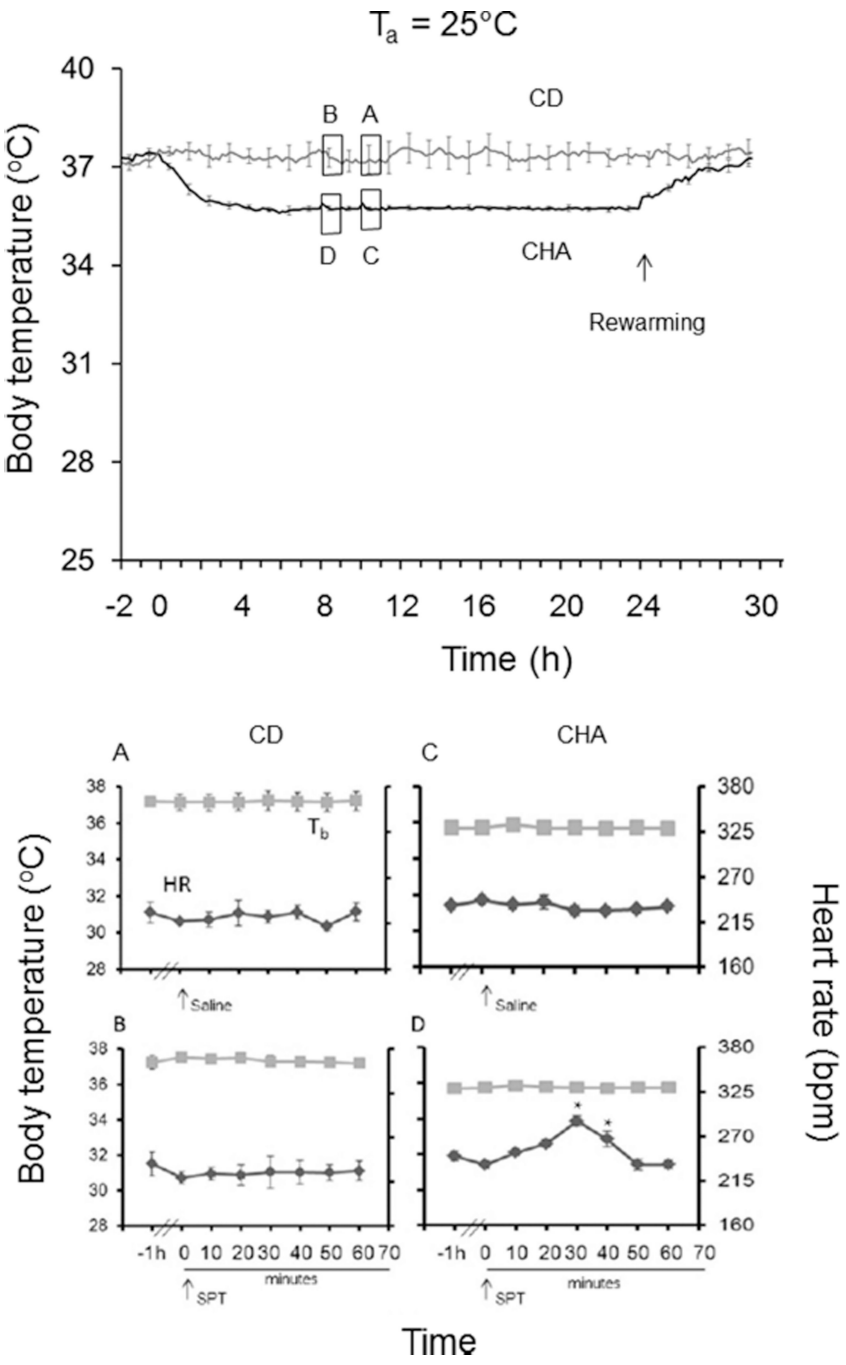

Figure 3.

Continuous CHA delivery via a SQ minipump at a rate equivalent to 1.0 mg/kg every 4 h at a T_a of 25 °C produces stable hypothermia (top). 8-SPT (25 mg/kg, IP) delivered 8 h after the onset of CHA delivery (B and D) increases heart rate without affecting body temperature (D). 8-SPT vehicle (saline) delivered 1 h later had no effect (A and C) (★$p < 0.05$; $n = 5$). The symbol and line style defined as T_b in panel A denotes T_b in panels B–D. The symbol and line style defined as HR in panel A denotes HR in panels B–D.

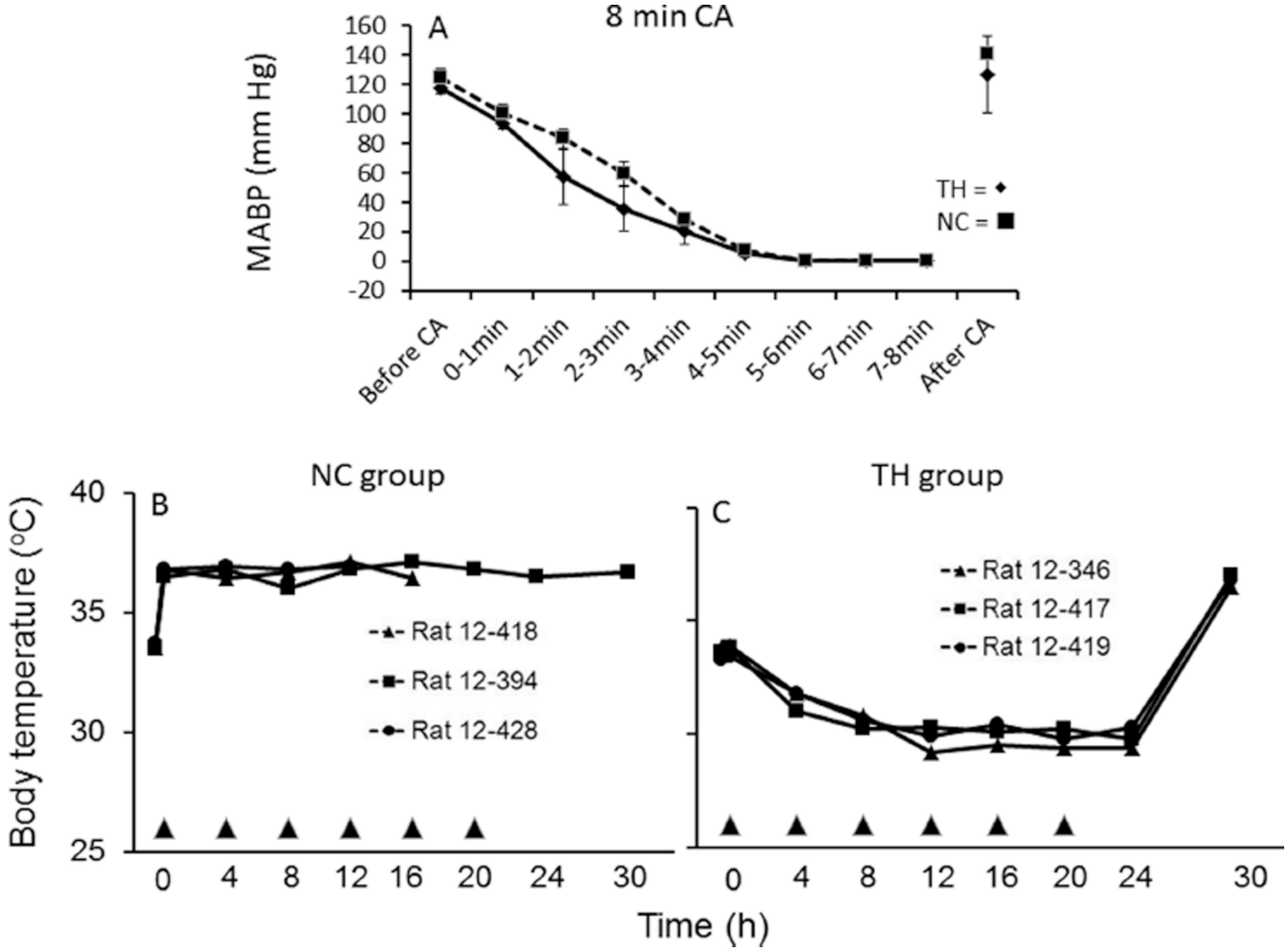

Figure 4.

(A) Mean arterial blood pressure (MABP) before, during, and after induction of asphyxial cardiac arrest for 8 min was similar in rats subsequently assigned to therapeutic hypothermia (TH, ◆) ($n = 3$) or normothermic control (NC, ■) ($n = 3$) groups. (B) T_b in NC and (C) T_b in TH-treated rats. Only one rat in the NC group survived for 7 days.

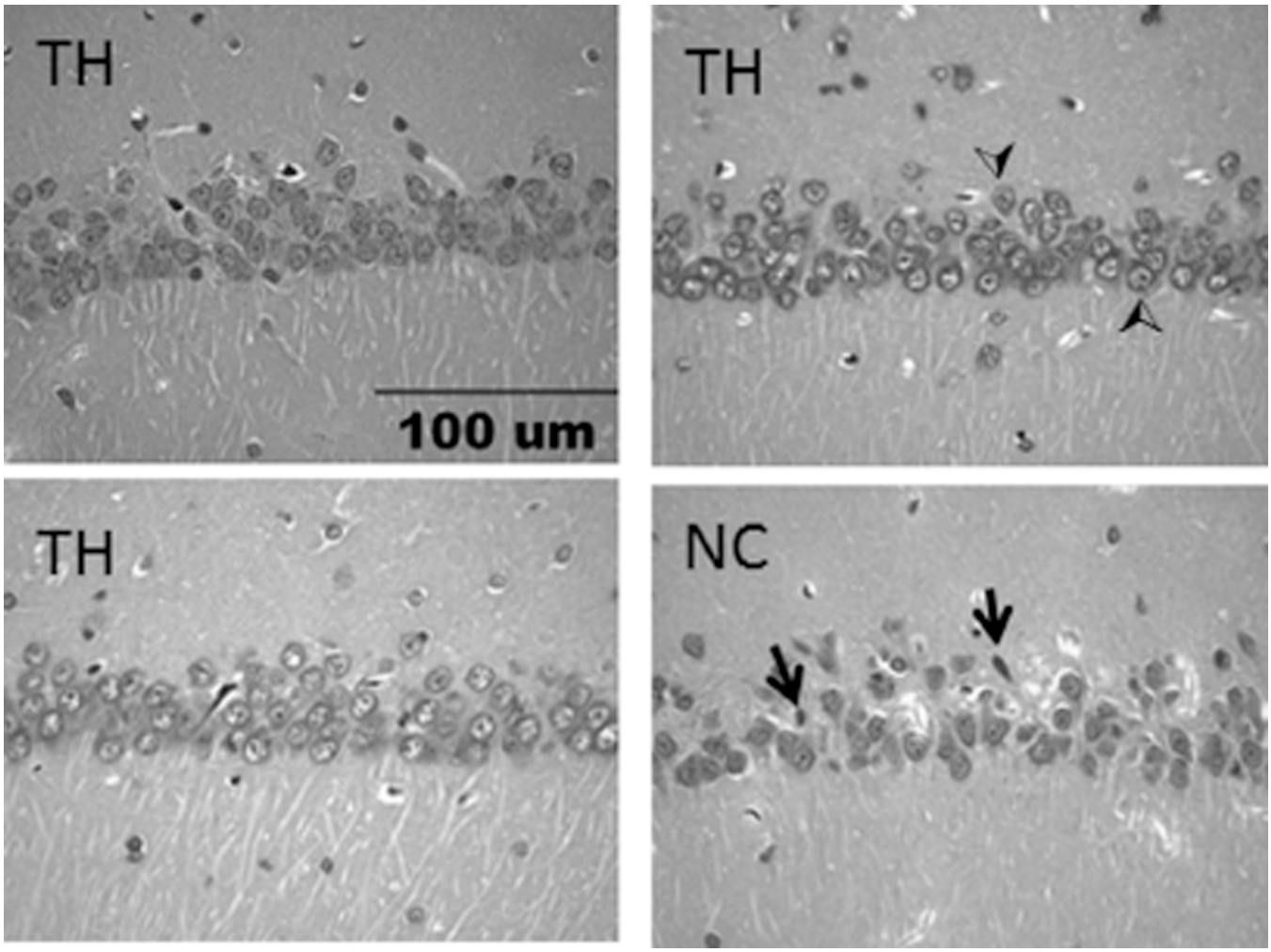

Figure 5.

Representative images showing the histopathology of pyknotic (ischemic, arrows) and healthy (normal, arrowheads) neurons in the CA1 hippocampal region of rats 8 days after asphyxial CA for 8 min in TH and NC groups. The scale bar is 100 μm.

Table 1

time from ROSC (min)	criteria for inclusion
2	ROSC within 120 s
30	blood gases stable and within normal ranges, base excess of >0
30–40	MABP ≥ 80 mmHg
60–70	$T_b \geq 33$ °C
70	comatose (unresponsive to toe pinch)