

Adenosine Receptors in Targeted Temperature Management: A New Approach to Cardiac Arrest Therapy

Neeshi Hullavarad¹, DeAnna Lowden², Hailey Baker², Bernard Laughlin²

¹Bates College, Lewiston, Maine 04240; ²University of Alaska, Fairbanks, Alaska, 99709

This review examines the combined use of the A₁ adenosine receptor (A₁AR) agonist, N⁶-cyclohexyladenosine (CHA), and the adenosine receptor antagonist, 8-(p-sulfophenyl)theophylline (8-SPT), as a proposed method of targeted temperature management (TTM) for cardiac arrest patients. TTM consists of central inhibition of thermogenesis as well as other body surface cooling mechanisms using the A₁AR. Neurological damage caused by cardiac arrest can kill or significantly impair life quality, however, studies of hibernation biology reveal fundamental mechanisms that can be applied to induce hypothermia by attenuating thermogenesis and limiting brain damage. Central activation of adenosine receptors by CHA in the solitary nucleus (NTS) inhibits thermogenesis. Unfortunately, this also results in unwanted cardiovascular effects in the periphery. For this reason, CHA can be administered in tandem with 8-SPT, which cannot cross the blood-brain barrier, to block adenosine receptor activation in the periphery. This review also includes an analysis of the effects of CHA and 8-SPT in the central nervous system (CNS) and peripheral nervous system (PNS). This novel dual administration of A₁AR ligands has effectively reached targeted temperatures in rats, guinea pigs, and hamsters, with the hope of being used in clinical cardiac arrest therapy.

Abbreviations: N⁶-cyclohexyladenosine - CHA; 8-(p-sulfophenyl)theophylline - 8-SPT; Targeted Temperature Management - TTM; Nucleus of the solitary tract – NTS; BBB – Blood Brain Barrier

Keywords: Hypoxic Ischemic Brain Injury; Cardiac Arrest; Adenosine; Targeted Temperature Management; CHA; 8-SPT; NTS

Introduction

More than 500,000 individuals in the United States suffer from cardiac arrest each year, with an estimated 350,000 cardiac arrests occurring outside of the hospital (Meaney et al., 2013; Benjamin et al., 2019). Cardiac arrest is associated with high in-hospital mortality and poor neurological outcome. In 2018, only 8.2% of adults that suffered an out-of-hospital cardiac arrest treated by emergency medical services survived with good functional status at hospital discharge (cerebral performance index of 1 or 2)(Virani et al., 2020). Cerebral performance scores of 3, 4, and 5 describe “severe neurological disability, persistent vegetative state, or death” (Reynolds and Soar, 2014). Hence, the majority of surviving patients must

endure devastating neurological injury, which can drastically reduce life quality for patients and their families. Neurological damage by cardiac arrest is a result of insufficient oxygen to the brain, resulting in irreversible neuronal damage known as hypoxic ischemic brain injury (Sekhon et al., 2017). During total circulatory arrest, adenosine triphosphate (ATP) production ceases and cellular integrity is compromised, triggering glutamate release (Geocadin et al., 2008). Excessive glutamate release activates N-methyl-D-aspartate (NMDA) receptors, flooding cells with extracellular calcium ions, and causing many issues. These issues include the formation of reactive oxygen species, potentially leading to lipid peroxidation, protein oxidation, and DNA

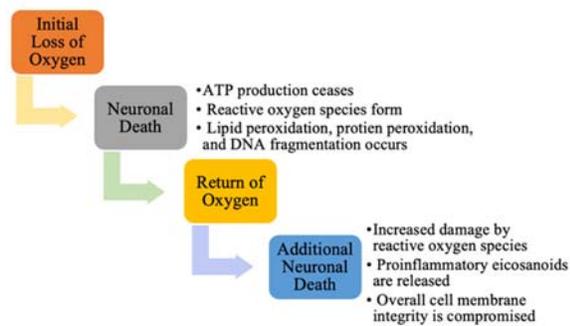


Figure 1. Neuronal damage caused by cardiac arrest during initial loss and return of oxygen.

fragmentation, which are all known causes of cell death (Geocadin et al., 2008). Paradoxically, the return of blood flow, also known as reperfusion, can lead to cerebral reperfusion injury which further kills neurons (Sekhon et al., 2017; Cowled and Fitridge, 2011). A chain of reactions produced by highly reactive species such as O_2^- and the OH radical ($OH\cdot$) consequently furthers cellular membrane lipid peroxidation, releasing proinflammatory eicosanoids, threatening cellular membrane integrity and leading to more cell death (Geocadin et al., 2008; Cowled and Fitridge, 2011). Essentially, while the initial cease in blood flow during cardiac arrest threatens cells, the reintroduction of oxygen exacerbates injury.

Brain injury is also not homogenous (Sharma et al., 2011; Geocadin et al., 2008). There is some discrepancy as to which parts of the brain are the most vulnerable. In a swine cardiac arrest model, the thalamus was found to be the most susceptible to damage compared to the cortex, hypothalamus, hippocampus, and brain stem (Sharma et al., 2011). On the other hand, Geocadin et al.'s (2008) review of human cardiac arrest studies claims the cortex is more vulnerable than the subcortical structures previously mentioned. Regardless, brain damage after cardiac arrest can result in memory and learning deficits, issues with motor control, and a lack of consciousness (Virani et al., 2020; Reynolds and Soar, 2014). This review examines the use of two A_1 adenosine receptor subtype (A_1AR) ligands, N^6 -cyclohexyladenosine (CHA) and 8-(p-sulfophenyl)theophylline (8-SPT), in targeted temperature management (TTM) as a

way to reduce neurological damage caused by oxygen deficiency from cardiac arrest. Discussion of TTM reveals a need for a centrally acting drug that inhibits shivering. This need is met by CHA which causes unwanted side effects in the periphery. 8-SPT can block the effects of CHA in the periphery when administered as a pretreatment. The combined use of these drugs shows promise as a cardiac arrest therapy.

Targeted Temperature Management

Current methods of mitigating brain injury caused by cardiac arrest include targeted temperature management (TTM), previously known as therapeutic hypothermia (Omairi and Pandey, 2020; Laughlin et al., 2018). TTM is a clinical protocol that cools the core body temperature to 32-34 °C from its average temperature of 36.1-37.2 °C and implements mechanisms to suppress thermogenesis (Omairi and Pandey, 2020; Saigal et al., 2015). Cerebral metabolism decreases by 8% with every 1 °C decrease in body temperature. Reduced cellular metabolic requirement protects neurons from damage after cardiac arrest while also decreasing cerebral blood flow, addressing the risk of cerebral reperfusion injury (Saigal et al., 2015). Widely used TTM practices include skin surface cooling via air and water, as well as core body cooling using methods such as cooled IV saline infusions, antipyretics, anesthetics, and intravascular catheters (Lundbye, 2012).

Shivering remains one of the most common complications of TTM. Shivering is a thermogenic reflex caused by the core body temperature decreasing below the hypothalamic set temperature point (Jain et al., 2018). Shivering is counterproductive to TTM because it increases cerebral metabolic demand by decreasing brain tissue oxygen tension. Brain tissue oxygen tension is a way to measure brain oxygen supply (Oddo et al., 2010). Low levels of brain tissue oxygen tension is associated with poor neurological outcomes. Lowering body temperature increases brown adipose tissue (BAT) activity which results in increased energy consumption and heat generation (Morrison,

2016). This is because BAT is a type of highly vascularized and metabolically active body fat that has increased amounts of mitochondria to generate heat (Shimizu et al., 2014; Chechi et al., 2013). Cutaneous vasoconstriction also poses an issue because it reduces skin heat conductance (Song and Lyden, 2012). Fortunately, activation of adenosine receptors in the nucleus of the solitary tract (NTS), located in the brain stem, can inhibit shivering, BAT activity, and cutaneous vasoconstriction (Tupone et al., 2013; Morrison, 2016). In addition to its central role in thermogenesis attenuation, the NTS is also known for receiving and integrating initial peripheral metabolic, gustatory, cardiovascular, and pulmonary signals (Morrison, 2016; Sekizawa et al., 2013; Tupone et al., 2013). The implications of the NTS in thermogenesis inhibition is of current interest in the field of hibernation biology.

Adenosine is found in the central nervous system (CNS) and peripheral nervous system (PNS) (Biaggioni et al., 1992; Sheth et al., 2014; Vallon and Oswald, 2009). Adenosine receptors are G-protein coupled receptors and are found as four subtypes within humans: A₁, A_{2A}, A_{2B}, and A₃ (Latini and Pedata, 2001; Sheth et al., 2014). Neuroprotective properties rest primarily with the A₁AR subtype which is widespread in the CNS (Boison, 2008). There is a high density of A₁ARs in the hippocampus, cortex, cerebellum, and NTS (Bisserbe et al., 1985; Sheth et al., 2014; Tupone et al., 2013). Activation of A₁ARs is known to play an inhibitory role in the CNS (Von Lubitz et al., 1993; Bjorness and Greene, 2009). Cellular mechanisms of A₁AR activation include neuron hyperpolarization to decrease electrical transmission and neuronal Ca²⁺ uptake inhibition, which attenuates neurotransmitter release in the presynaptic terminal (Latini and Pedata, 2001; Aerde et al., 2015). These effects result in reduced energy expenditure and decreased cellular metabolism which is a great benefit for TTM.

Torpor is an adaptive state of dormancy during periods of expected famine (Withers et al., 2008; Boyer and Barnes, 1999). When animals are exposed to harsh cold temperatures, this state of dormancy is known as hibernation. Animals

also experience decreases in body temperature, metabolic demand, heart rate, blood pressure, oxygen consumption, and brain activity (Boyer and Barnes, 1999; Jinka et al., 2011). Activating A₁ARs in the brain is necessary to induce torpor in hibernating species, presumably through the NTS which likely senses changes in metabolic, not thermal, parameters (Morrison, 2016). More importantly, torpor-like states have been induced in animals that do not naturally hibernate such as rats and guinea pigs using the A₁AR (BAT activity inhibition, decrease in core body temperature and shivering) (Shiomi and Tamura, 2000; Jinka et al., 2011; Tupone et al., 2013; Shimaoka et al., 2018). There have been many studies that have found that activation of A₁ARs, specifically by the agonist, CHA, can decrease core body temperature and induce torpor. Findings from those studies will be discussed in the next section.

CHA and Thermogenesis

CHA is a well-studied potential thermolytic agent for TTM. CHA is an A₁AR agonist, with some affinity for A₃AR, and a very weak affinity for A₂AR (Gao et al., 2003). Many studies demonstrate central CHA agonism of A₁AR effectively attenuating thermogenesis (Tupone et al., 2013; Shintani et al., 2005; Bailey et al., 2017; Laughlin et al., 2018; Jinka et al., 2015). CHA (5ul, 1mM, ICV) administered in male rats over 24 h resulted in a core body temperature of 26.7 ± 0.9 °C, compared to saline administration resulting in a core body temperature of only 37.2 ± 0.6 °C (Tupone et al., 2013). An increase in dose is inversely correlated with body temperature in Syrian hamsters, another hibernator (Shintani et al., 2005). In a range of 0.05-0.5 nmol, 0.5 nmol resulted in the largest decrease of body temperature rates of 31.26 ± 0.11 °C/465 minutes, while the 0.05 nmol dose only resulted in a decrease of 4.31 ± 0.29 °C/44 minutes. The same study also found that CHA effects were greater at colder ambient temperatures. Administration of 0.5 nmol CHA ICV decreased body temperature at a rate of 31.99 ± 0.10 °C/465 minutes at an ambient temperature of 5 °C whereas 25 °C only reduced

body temperature by $6.39 \pm 0.25\text{C } ^\circ\text{C}/166.7$ minutes in Syrian hamsters.

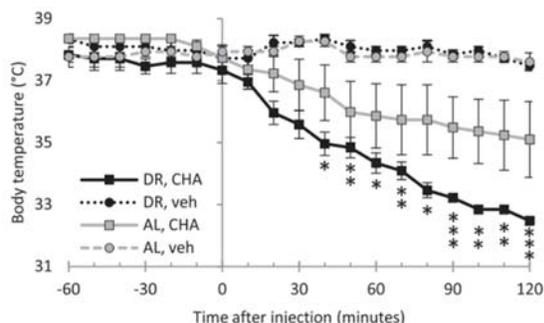


Figure 2. Body temperature versus time after CHA administration at an ambient temperature of 16 °C. Varying central A₁AR expression results in differing thermoinhibitory effects of CHA (0.5 mg/kg IP). Dietary restriction significantly sensitized rats to CHA compared to the AL group. (Bailey et al., 2017 - Reproduced with permission from the publisher).

While CHA has consistently shown promise as a thermolytic agent, individual response varies. This may have to do with A₁AR expression in central thermoregulatory regions. For instance, rats that were fed every other day (dietary restricted (DR)) showed increased A₁AR expression in the hypothalamus compared to rats fed ad libitum (AL) (Figure 2) (Bailey et al., 2017). This sensitized the DR rats to CHA and caused a greater decrease in body temperature. This also implicates the hypothalamus in the reduction of body temperature. DR rats also experienced less CHA variability than AL rats. These findings suggest that when individuals have similar amounts of central A₁AR, their response will also be similar. A dose of 1.0 mg/kg CHA given intraperitoneally resulted in AL animals reaching the target body temperature of 32 °C, however some animals over-cooled to 21 °C at an ambient temperature of 17.0 °C. To manage inter-individual variation, the thermolytic response to CHA was adjusted with conductive surface heating and cooling to achieve the desired target temperature (Bailey et al., 2017). This suggests that CHA in tandem with surface cooling can be an effective TTM therapy.

It should be noted that CHA tolerance can occur and is characteristic of other A₁AR agonists as well (Roman et al., 2008). Increasing

CHA dosage using intermittent injections or continuous IV titration in rats was unsuccessful because of acute tolerance development (Laughlin et al., 2018). Instead, continuous CHA (1.0 mg/kg IV) for 24 h in rats was sufficient to reach and maintain a target temperature between 32-34 °C with real-time conductive cooling to prevent overcooling. This standardized the responses of individual animals without causing any adverse effects (Laughlin et al., 2018). This suggests that CHA administration in addition to surface cooling or ambient temperature control can potentially be used for TTM in humans. On the other hand, even though CHA effectively reduces body temperature, it has not been used clinically because of its cardiovascular side effects (Jinka et al., 2015).

Mainly, CHA is known to have an inhibitory effect on heart rate but there is discrepancy about its effect on blood pressure. The following studies outline some of these findings. CHA (0.25 mg/[kg·h]) was administered to rats by continuous IV infusion and produced slower than normal heart rate, also known as bradycardia, within 30 min (Laughlin et al., 2018). A starting heart rate of between 300-400 beats per minute (bpm) decreased to 89 ± 4 bpm over 24 h (362 ± 9 bpm). In rats with normal blood pressure, ICV (0.01, 0.05, 0.1 mg) and IV (0.1, 0.5, 1.0 mg/kg) CHA decreased arterial pressure and heart rate in a dose-dependent manner (Stella et al., 1993). CHA given at 1 pmol ICV decreased hamster heart rate by $84 \pm 4\%$ at 3 min and remained near that value for greater than 5 min (Proctor et al., 1991). Systemic blood pressure decreased from 113 ± 4 mmHg to $86 \pm 5\%$ at 3 min and remained near 80% of control for greater than 15 min. Similar findings have been reported in ICV (5ul, 1mm) injections of CHA in rats as well (Tupone et al., 2013). The rats were kept in a cooled environment of 15 °C. Heart rate was 171 ± 42 bpm after CHA administration and 361 ± 15 bpm after saline administration. The reduced heart rate was maintained for 6 h. Interestingly, CHA increased mean arterial pressure by 39.5 ± 6 mmHg ($p = 0.003$). This contrasts with other findings. It is important to note that an increase in blood pressure was only seen when the animals were

Table 1. CHA decreases body temperature in rodents.

Source	Animal	Mode of CHA administration	Dose	Ambient Temperature (°C)	Approximate Decrease in Animal Body Temperature (°C)
Futatsuki <i>et al.</i> , 2018	Mouse	IP	200 nmol	21 ± 1	10
Zarrindast <i>et al.</i> , 1993	Mouse	IP	0.1 mg/kg	24 ± 0.2	2.4
Zarrindast <i>et al.</i> , 1993	Mouse	IP	0.25 mg/kg	24 ± 0.2	3.1
Bailey <i>et al.</i> , 2017	Rat	IP	0.5 mg/kg	16	2.5
Bailey <i>et al.</i> , 2017	Rat	IP	1.0 mg/kg	16	8
Jinka <i>et al.</i> , 2015	Rat	IP	1.0 mg/kg	16	5
Futatsuki <i>et al.</i> , 2018	Mouse	ICV	0.02 nmol	21 ± 1	5
Futatsuki <i>et al.</i> , 2018	Mouse	ICV	0.04 nmol	21 ± 1	7
Tupone <i>et al.</i> , 2013	Rat	ICV	5µl, 1mM	15	10.5
Shimaoka <i>et al.</i> , 2018	Rat	ICV	20 nmol	4*	22**
Laughlin <i>et al.</i> , 2018	Rat	IV	0.25 mg/(kg·h)	21.1±0.3	4.5
Bailey <i>et al.</i> , 2017	Rat	IV	0.25 mg/(kg·h)	16 to 32	6

Note. Various modes of CHA administration such as IP, ICV, and IV affect decrease in body temperature and is a function of ambient temperature. There is a dose dependent decrease in body temperature. Lower ambient temperatures with similar dosages resulted in greater decreases in body temperature.

Abbreviations: IV – Intravenous; ICV – Intracerebroventricular; IP – Intraperitoneal

*Rats were moved to an ambient temperature of 22 °C if body temperature dropped to 15 °C

**Lowest allowable body temperature was 15 °C. Required 312 ± 88 minutes for this value to be reached

cooled. As previously mentioned, hypothermia elicits cutaneous vasoconstriction, and these results could potentially be attributed to that. Some of these cardiovascular effects have also been seen in non-cooled primates. IV administration of CHA (cumulative 0.01-1.0 mg/kg) in cynomolgus monkeys resulted in dose-dependent decreases in heart rate and mean arterial blood pressure (Coffin and Spealman, 1987). These findings are pertinent because heart rate is widely accepted as a proxy for metabolic rate (Laughlin *et al.*, 2018; Currie *et al.*, 2014). While bradycardia during TTM indicates positive outcomes, severe bradycardia can compromise organ perfusion (Parish *et al.*, 2018). CHA in the cardiovascular system could potentially block the A₁ARs necessary for cardiac function once the patient's circulation has returned. Therefore, there is a balance that must be achieved when CHA is administered in the periphery. Low blood pressure, also known as hypotension, after the spontaneous return of circulation following cardiac arrest is a predictor of poor neurological outcomes and has been found to be an independent predictor of mortality (Geocadin *et al.*, 2008; Trzeciak *et al.*, 2009; Kilgannon *et al.*, 2008; Laughlin *et al.*, 2018).

About 12-81% of cardiac arrest patients experience acute kidney injury. (Spoelstra-de Man and Oudemans-van Straaten, 2019; Rundgren *et al.*, 2019). This wide percentage range is a result of studies using different definitions for acute kidney injury and patient selection (Rundgren *et al.*, 2019) Acute kidney injury is associated with severe neurological outcomes, increased dialysis requirements and time in the hospital, and greater mortality (Spoelstra-de Man and Oudemans-van Straaten, 2019). It is inconclusive if TTM reduces acute renal injury after cardiac arrest. That being said, adenosine plays a significant role in the renal system and there is value to examine it here. While A₁AR activation generally leads to vasodilation in most tissues, it causes vasoconstriction in the renal cortex to meet metabolic restraints (Vallon and Oswald, 2009). Blood flow to the renal cortex forms an ultrafiltrate and increases the metabolic demand for tubular electrolyte transport. Essentially, glomerular filtration rate becomes too high for renal blood flow and it is necessary to decrease the ratio between the two. In contrast to the renal cortex, the renal medulla still needs adequate blood flow (Vallon and Oswald, 2009).

Adenosine works with the renin-angiotensin system to cause vasoconstriction in the renal cortex and vasodilation in the renal medulla (Biaggioni, 1992; Vallon and Oswald, 2009). In isolated perfused rat kidneys, CHA (0.001 to 0.1 μ M) caused concentration-dependent vasoconstriction and relative vasodilation at higher concentrations, somewhat mimicking adenosine (Rossi et al., 1987). CHA (1 μ M) has been shown to decrease glomerular filtration rate in perfused rat kidneys (Murray and Churchill, 1984). CHA also increased afferent arteriolar resistance but decreased efferent arteriolar resistance. It is clear that A₁ARs play an essential role in the kidneys, and likewise, are necessary for other organ systems as well. However, there is an impending need to prevent A₁AR agonism in the heart to effectively cool the body. This can realistically be accomplished in a clinical setting by using an antagonist that only acts in the periphery.

8-SPT in the Periphery

The A₁AR antagonist, 8-SPT, can block the effects of CHA in the PNS. 8-SPT is a non-specific A₁AR and A_{2A}AR antagonist that does not readily cross the blood brain barrier (BBB) due to its polarity (Evoniuk et al., 1987). For this reason, 8-SPT has little to no effect on the brain when administered into the periphery. Few studies have examined the effects of sole 8-SPT administration, but they provide valuable information about how 8-SPT impacts body temperature and heart rate. In fasted, torpid, mice, 8-SPT (50 mg/kg, subcutaneous) did not cause a change in body temperature even after an hour of infusion (8-SPT = 28.1 \pm 1.6 $^{\circ}$ C, saline = 29.3 \pm 2.2 $^{\circ}$ C) (Iloff and Swoap, 2012). This lack of effect from peripheral administration was contrasted to the 8-SPT given intracerebroventricularly. ICV 8-SPT (10 μ g in 5 μ l vehicle) increased body temperature in comparison to the phosphate-buffered saline solution by 33 minutes after infusion began (saline = 31.9 \pm 1.1 $^{\circ}$ C, 8-SPT = 34.3 $^{\circ}$ C \pm 1.3 $^{\circ}$ C, P < 0.05). Cardiovascular effects were also measured in the same study. 8-SPT (50 mg/kg, subcutaneous) in the periphery did not cause a significant change in heart rate when compared to

the saline solution (8-SPT = 429 \pm 95 bpm, saline = 399 \pm 79 bpm), but ICV 8-SPT (10 μ g in 5 μ l vehicle) increased heart rate (saline = 380 \pm 25 bpm, 8-SPT = 602 \pm 60 bpm, P < 0.05). While sole peripheral 8-SPT administration does not impact body temperature or heart rate, 8-SPT antagonizes cardiovascular effects of A₁AR agonists including CHA (Thomas and Spyer, 1996; Joosen et al., 2004; Sidi et al., 1994). A pretreatment of 8-SPT (50 mg/kg) in rats infused with adenosine (1.74 mM arterial adenosine) reduced hypotension caused by adenosine (Evoniuk et al., 1987). The administration of 8-SPT (20 mg/kg, IV) after the adenosine analog 2-chloroadenosine (30 mg/kg IV) reduced bradycardia and kept arterial blood pressure from decreasing significantly (Thomas et al., 1994). Thus, when administered as a pretreatment, 8-SPT can be used to localize the effects of CHA exclusively to the CNS without introducing additional peripheral effects.

Combined Effects of CHA and 8-SPT

The dual administration of CHA and 8-SPT has been successful in inducing hypothermia and mitigating cardiovascular effects. Consistent with other studies, a study by Jinka et al. (2015) using rats found that lower ambient temperatures resulted in lower CHA-induced body temperatures. When CHA was delivered continuously at a dose equivalent to 1.0 mg/kg every 4, an ambient temperature of 16 $^{\circ}$ C caused the average minimum body temperature to become 29.3 \pm 0.3 $^{\circ}$ C. The average minimum body temperature was 35.6 \pm 0.1 $^{\circ}$ C at an ambient temperature of 25 $^{\circ}$ C. The authors believed this difference in response was because body heat dissipated more with the lower ambient temperature in addition to reduced thermogenesis caused by CHA. Even more, 8-SPT (25 mg/kg) was administered 8 h after onset of continuous CHA administration. This did not have a significant effect on body temperature but did increase heart rate at ambient temperatures of 16 $^{\circ}$ C and 25 $^{\circ}$ C, less so with the latter. Essentially, 8-SPT had an enhanced effect at lower ambient temperatures. This process was an effective method of temporally controlling when target

temperatures were met. Jinka et al. went one step further and created a model of asphyxial cardiac arrest. At an ambient temperature of 16 °C, 25 mg/kg 8-SPT was injected every four hours and was followed 15 minutes later by an injection of 1 mg/kg CHA. Rats given this treatment were cooled from a starting body temperature of 33.5 ± 0.1 °C to temperatures ranging from 31.0–29.2 °C. The average minimum body temperature was 29.7 ± 0.3 °C. Control rats that were not treated or cooled had a body temperature ranging from 36.2 °C to 37.3 °C. While cardiac arrest caused damage to hippocampal CA-1 neurons in all rats, animals given the treatment had significantly less injury than the control group. Therefore, TTM effectively preserved some neurons in this study. Additionally, the rats were successfully rewarmed by discontinuing the treatment and placing them in warmer ambient temperatures (Jinka et al., 2015). Ultimately, these findings indicate that the dual administration of CHA and 8-SPT with ambient temperature control is effective at modulating body temperature and reaching TTM goals in a cardiac arrest model. Successful rewarming of animals has also been seen in other studies.

While this TTM therapy shows great promise, it is important to note a few issues with this A₁AR agonist/antagonist model. Firstly, while 8-SPT is not BBB permeable, cardiac arrest may compromise BBB integrity, allowing 8-SPT to antagonize the effects of CHA in the brain (Sharma et al., 201; Guo et al., 2018). This may interfere in the goals of TTM simply because CHA won't be able to bind to A₁ARs. Secondly, A₁AR agonists, including CHA, are well known to protect against seizures by increasing seizure threshold and decreasing occurrence (Zeraati et al., 2006; Murray et al., 1985). Adenosine receptor antagonists, such as theophylline and aminophylline, have reportedly countered seizure protective properties of A₁AR agonists at higher doses (Borowicz et al., 1993; Murray et al., 1985). This is notable because seizures are common after cardiac arrest and the high metabolic demand caused by the increased neuronal activity can cause further damage in an already vulnerable brain. The administration of other anticonvulsant drugs alongside 8-SPT and CHA may be a solution and needs further study.

Conclusion

In summary, cardiac arrest is a severe medical emergency. Depletion of the brain's oxygen supply frequently results in neuronal damage. The reintroduction of oxygen after cardiac arrest can cause cerebral reperfusion injury and worsen neurological outcomes. Cooling the body after the cardiac arrest can reduce the injury. However, therapeutic cooling in non-hibernators elicits counterproductive reflexes such as shivering, BAT thermogenesis, and cutaneous vasoconstriction. Agonizing A₁ARs in the brain using CHA mimics neural mechanisms of natural hibernators.

While CHA can inhibit thermogenesis, it comes with cardiovascular side effects. For this reason, the A₁AR antagonist 8-SPT can be used to block CHA in the periphery. Treatment of 8-SPT followed by CHA, along with surface or ambient temperature control, can be used for TTM and mitigation of the damage caused by cardiac arrest. It also allows target temperatures to be met with temporal control. It should be noted that A₁AR exists in the periphery too, such as in the renal system, and antagonizing these receptors can potentially block endogenous adenosine from activating them. Till date, there are no reports on the use of CHA and 8-SPT in humans. Successful TTM in human trials is dependent on further study of the dual administration of CHA and 8-SPT in other species. Lastly, the A₁AR agonist/antagonist model may provide insight on treating other conditions.

Acknowledgements

We would like to thank our mentor, Professor Kelly Drew, for her guidance and insight on this project, as well as Thaddeus Gunther for his review of the manuscript. We would also like to thank the following sources of funding: Bates College Center for Purposeful Work, Alaska Center for Innovation, Commercialization, and Entrepreneurship, the University of Alaska Undergraduate Research and Scholarly Activity, Alaska NASA EPSCoR and the Clinical Trials Reporting Program.

Corresponding Author

Bernard Laughlin, D.O
University of Alaska
902 N. Koyukuk
P.O. Box 757000
Fairbanks, AK 99775-7000
bbaughli@alaska.edu

References

- Bailey IR, Laughlin B, Moore LA, Bogren LK, Barati Z, Drew KL (2017) Optimization of Thermolytic Response to A(1) Adenosine Receptor Agonists in Rats. *J Pharmacol Exp Ther* 362:424-430.
- Benjamin EJ et al. (2019) Heart Disease and Stroke Statistics —2019 Update: A Report From the American Heart Association. *Circulation* 139:e56-e528.
- Biaggioni I (1992) Contrasting excitatory and inhibitory effects of adenosine in blood pressure regulation. *Hypertension (Dallas, Tex : 1979)* 20:457-465.
- Bisserbe JC, Patel J, Marangos PJ (1985) Autoradiographic localization of adenosine uptake sites in rat brain using [3H]nitrobenzylthioinosine. *J Neurosci* 5:544.
- Bjorness TE, Greene RW (2009) Adenosine and sleep. *Curr Neuropharmacol* 7:238-245.
- Boison D (2008) Adenosine as a neuromodulator in neurological diseases. *Curr Opin Pharmacol* 8:2-7.
- Borowicz K, Kozicka M, Kleinrok Z, Czuczwar SJ (1993) Influence of aminophylline and 8-(p-sulfophenyl)theophylline on the anticonvulsive action of diphenylhydantoin, phenobarbital, and valproate against maximal electroshock-induced convulsions in mice. *J Neural Transm* 93:157-163.
- Boyer BB, Barnes BM (1999) Molecular and Metabolic Aspects of Mammalian Hibernation: Expression of the hibernation phenotype results from the coordinated regulation of multiple physiological and molecular events during preparation for and entry into torpor. *BioScience* 49:713-724.
- Cechi K, Carpentier AC, Richard D (2013) Understanding the brown adipocyte as a contributor to energy homeostasis. *Trends Endocrinol Metab* 24:408-420.
- Coffin VL, Spealman RD (1987) Behavioral and cardiovascular effects of analogs of adenosine in cynomolgus monkeys. *J Pharmacol Exp Ther* 241:76-83.
- Cowled P, Fitridge R (2011) Pathophysiology of Reperfusion Injury. In: *Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists [Internet]* (Fitridge R, Thompson M, eds): Adelaide (AU): University of Adelaide Press.
- Currie SE, Körtner G, Geiser F (2014) Heart rate as a predictor of metabolic rate in heterothermic bats. *J Exp Biol* 217:1519.
- Evoniuk G, von Borstel RW, Wurtman RJ (1987) Antagonism of the cardiovascular effects of adenosine by caffeine or 8-(p-sulfophenyl)theophylline. *J Pharmacol Exp Ther* 240:428.
- Gao ZG, Blaustein JB, Gross AS, Melman N, Jacobson KA (2003) N6-Substituted adenosine derivatives: selectivity, efficacy, and species differences at A3 adenosine receptors. *Biochem Pharmacol* 65:1675-1684.
- Geocadin RG, Koenig MA, Jia X, Stevens RD, Peberdy MA (2008) Management of brain injury after resuscitation from cardiac arrest. *Neurologic clinics* 26:487-ix.
- Guo Q, Yang J, Hu Z, Xiao Y, Wu X, Bradley J, Peberdy Mary A, Ornato J, Mangino M, Tang W (2018) Abstract 253: Increased Blood Brain Barrier Permeability After Successful Resuscitation in a Rat Model of Cardiac Arrest. *Circulation* 138:A253-A253.
- Iliff BW, Swoap SJ (2012) Central adenosine receptor signaling is necessary for daily torpor in mice. *Am J Physiol Regul Integr Comp Physiol* 303:R477-484.
- Jain A, Gray M, Slisz S, Haymore J, Badjatia N, Kulstad E (2018) Shivering Treatments for Targeted Temperature Management: A Review. *J Neurosci Nurs* 50:63-67.
- Jinka TR, Combs VM, Drew KL (2015) Translating drug-induced hibernation to therapeutic hypothermia. *ACS Chem Neurosci* 6:899-904.
- Jinka TR, Tøien Ø, Drew KL (2011) Season Primes the Brain in an Arctic Hibernator to Facilitate Entrance into Torpor Mediated by

- Adenosine A₁ Receptors. *J Neurosci* 31:10752.
- Joosen MJ, Bueters TJ, van Helden HP (2004) Cardiovascular effects of the adenosine A₁ receptor agonist N⁶-cyclopentyladenosine (CPA) decisive for its therapeutic efficacy in sarin poisoning. *Arch Toxicol* 78:34-39.
- Kilgannon JH, Roberts BW, Reihl LR, Chansky ME, Jones AE, Dellinger RP, Parrillo JE, Trzeciak S (2008) Early arterial hypotension is common in the post-cardiac arrest syndrome and associated with increased in-hospital mortality. *Resuscitation* 79:410-416.
- Latini S, Pedata F (2001) Adenosine in the central nervous system: release mechanisms and extracellular concentrations. *J Neurochem* 79:463-484.
- Laughlin BW, Bailey IR, Rice SA, Barati Z, Bogren LK, Drew KL (2018) Precise Control of Target Temperature Using N(6)-Cyclohexyladenosine and Real-Time Control of Surface Temperature. *Ther Hypothermia Temp Manag* 8:108-116.
- Lundbye MWPJB (2012) Hypothermia: How to Cool. In: *Therapeutic Hypothermia After Cardiac Arrest: Clinical Application and Management* (Lundbye JB, ed): Springer-Verlag London.
- Meaney Peter A, Bobrow Bentley J, Mancini Mary E, Christenson J, de Caen Allan R, Bhanji F, Abella Benjamin S, Kleinman Monica E, Edelson Dana P, Berg Robert A, Aufderheide Tom P, Menon V, Leary M (2013) Cardiopulmonary Resuscitation Quality: Improving Cardiac Resuscitation Outcomes Both Inside and Outside the Hospital. *Circulation* 128:417-435.
- Morrison SF (2016) Central neural control of thermoregulation and brown adipose tissue. *Auton Neurosci* 196:14-24.
- Murray RD, Churchill PC (1984) Effects of adenosine receptor agonists in the isolated, perfused rat kidney. *Am J Physiol* 247:H343-348.
- Murray TF, Sylvester D, Schultz CS, Szot P (1985) Purinergic modulation of the seizure threshold for pentylenetetrazol in the rat. *Neuropharmacology* 24:761-766.
- Oddo M, Frangos S, Maloney-Wilensky E, Andrew Kofke W, Le Roux PD, Levine JM (2010) Effect of Shivering on Brain Tissue Oxygenation During Induced Normothermia in Patients With Severe Brain Injury. *J Neurocrit Care* 12:10-16.
- Omairi A, Pandey S (2020) Targeted Hypothermia Temperature Management. In: *StatPearls* [Internet]: Treasure Island (FL): StatPearls Publishing.
- Parish DC, Goyal H, Dane FC (2018) Mechanism of death: there's more to it than sudden cardiac arrest. *J Thorac Dis* 10:3081-3087.
- Proctor KG, Stojanov I, Bealer SL (1991) Activation of brain adenosine receptors evokes vasodilation in skin arterioles. *Am J Physiol* 261:H457-462.
- Reynolds EC, Soar J (2014) How are cerebral performance category scores measured for audit and research purposes? *Resuscitation* 85:e73-e74.
- Roman V, Keijsers JN, Luiten PG, Meerlo P (2008) Repetitive stimulation of adenosine A₁ receptors in vivo: changes in receptor numbers, G-proteins and A₁ receptor agonist-induced hypothermia. *Brain Res* 1191:69-74.
- Rossi NF, Churchill PC, Jacobson KA, Leahy AE (1987) Further characterization of the renovascular effects of N⁶-cyclohexyladenosine in the isolated perfused rat kidney. *J Pharmacol Exp Ther* 240:911.
- Rundgren M, Ullén S, Morgan MPG, Glover G, Cranshaw J, Al-Subaie N, Walden A, Joannidis M, Ostermann M, Dankiewicz J, Nielsen N, Wise MP (2019) Renal function after out-of-hospital cardiac arrest; the influence of temperature management and coronary angiography, a post hoc study of the target temperature management trial. *Crit Care* 23:163.
- Saigal S, Sharma JP, Dhurwe R, Kumar S, Gurjar M (2015) Targeted temperature management: Current evidence and practices in critical care. *Indian journal of critical care medicine : peer-reviewed, official publication of Indian Society of Critical Care Medicine* 19:537-546.
- Sekhon MS, Ainslie PN, Griesdale DE (2017) Clinical pathophysiology of hypoxic ischemic brain injury after cardiac arrest: a "two-hit" model. *Crit care (London, England)* 21:90-90.
- Sekizawa S-I, Horwitz BA, Horowitz JM, Chen C-Y (2013) Protection of signal processing at low temperature in baroreceptive neurons in

- the nucleus tractus solitarius of Syrian hamsters, a hibernating species. *Am J Physiol Regul Integr Comp Physiol* 305:R1153-R1162.
- Sharma HS, Miclescu A, Wiklund L (2011) Cardiac arrest-induced regional blood-brain barrier breakdown, edema formation and brain pathology: a light and electron microscopic study on a new model for neurodegeneration and neuroprotection in porcine brain. *J Neural Transm* 118:87-114.
- Sheth S, Brito R, Mukherjea D, Rybak LP, Ramkumar V (2014) Adenosine receptors: expression, function and regulation. *Int J Mol Sci* 15:2024-2052.
- Shimaoka H, Kawaguchi T, Morikawa K, Sano Y, Naitou K, Nakamori H, Shiina T, Shimizu Y (2018) Induction of hibernation-like hypothermia by central activation of the A1 adenosine receptor in a non-hibernator, the rat. *J Physiol Sci* 68:425-430.
- Shimizu I, Aprahamian T, Kikuchi R, Shimizu A, Papanicolaou KN, MacLauchlan S, Maruyama S, Walsh K (2014) Vascular rarefaction mediates whitening of brown fat in obesity. *J Clin Invest* 124:2099-2112.
- Shintani M, Tamura Y, Monden M, Shiomi H (2005) Characterization of N6-Cyclohexyladenosine-Induced Hypothermia in Syrian Hamsters. *J Pharmacol Sci* 97:451-454.
- Shiomi H, Tamura Y (2000) [Pharmacological aspects of mammalian hibernation: central thermoregulation factors in hibernation cycle]. *Nihon Yakurigaku Zasshi* 116:304-312.
- Sidi A, Wesley R, Barrett R, Rush W, Belardinelli L (1994) Cardiovascular effects of a non-xanthine-selective antagonist of the A1 adenosine receptor in the anaesthetised pig: pharmacological and therapeutic implications. *Cardiovasc Res* 28:621-628.
- Song SS, Lyden PD (2012) Overview of therapeutic hypothermia. *Curr Treat Options Neurol* 14:541-548.
- Spoelstra-de Man AME, Oudemans-van Straaten HM (2019) Acute kidney injury after cardiac arrest: the role of coronary angiography and temperature management. *Crit Care* 23:193.
- Stella L, Berrino L, Maione S, de Novellis V, Rossi F (1993) Cardiovascular effects of adenosine and its analogs in anaesthetized rats. *Life Sci* 53:755-763.
- Thomas T, Elnazir BK, Marshall JM (1994) Differentiation of the peripherally mediated from the centrally mediated influences of adenosine in the rat during systemic hypoxia. *Exp Physiol* 79:809-822.
- Thomas T, Spyer KM (1996) The role of adenosine receptors in the rostral ventrolateral medulla in the cardiovascular response to defence area stimulation in the rat. *Exp Physiol* 81:67-77.
- Trzeciak S, Jones AE, Kilgannon JH, Milcarek B, Hunter K, Shapiro NI, Hollenberg SM, Dellinger RP, Parrillo JE (2009) Significance of arterial hypotension after resuscitation from cardiac arrest*. *Crit Care Med* 37.
- Tupone D, Madden CJ, Morrison SF (2013) Central activation of the A1 adenosine receptor (A1AR) induces a hypothermic, torpor-like state in the rat. *J Neurosci* 33:14512-14525.
- Vallon V, Osswald H (2009) Adenosine receptors and the kidney. *Handb Exp Pharmacol*:443-470.
- van Aerde KI, Qi G, Feldmeyer D (2015) Cell Type-Specific Effects of Adenosine on Cortical Neurons. *Cereb Cortex* 25:772-787.
- Virani SS et al. (2020) Heart Disease and Stroke Statistics —2020 Update: A Report From the American Heart Association. *Circulation* 141:e139-e596.
- Von Lubitz DK, Paul IA, Bartus RT, Jacobson KA (1993) Effects of chronic administration of adenosine A1 receptor agonist and antagonist on spatial learning and memory. *Eur J Pharmacol* 249:271-280.
- Withers PC, Cooper CE, Jørgensen SE, Fath BD (2008) Dormancy. In: *Encyclopedia of Ecology*, pp 952-957. Oxford: Academic Press.
- Zeraati M, Mirnajafi-Zadeh J, Fathollahi Y, Namvar S, Rezvani ME (2006) Adenosine A1 and A2A receptors of hippocampal CA1 region have opposite effects on piriform cortex kindled seizures in rats. *Seizure* 15:41-48.