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Hypothermia in mouse is caused by adenosine A_1 and A_3 receptor agonists and AMP via three distinct mechanisms

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Chemical compounds:
AMP
Adenosine 5'-monophosphate
CCPA
2-Chloro-N^6-cyclopentyladenosine
CHA
N^6-cyclohexyladenosine
Cl-ENBA
(±)-5'-chloro-5'-deoxy-N^6-*endo*-norbornyladenosine
CPA
N^6-cyclopentyladenosine
MRS5474
(1R,2R,3S,5S)-4-(2-chloro-6-((dicyclopropylmethyl)amino)-9H-purin-9-yl)bicyclo[3.1.0]hexane-2,3-diol
R-PIA
N^6-R-phenylisopropyladenosine
SPA
N^6-(p-sulfo-phenyl)adenosine

Keywords:
Hypothermia
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ABSTRACT

Small mammals have the ability to enter torpor, a hypothermic, hypometabolic state, allowing impressive energy conservation. Administration of adenosine or adenosine 5'-monophosphate (AMP) can trigger a hypothermic, torpor-like state. We investigated the mechanisms for hypothermia using telemetric monitoring of body temperature in wild type and receptor knock out (*Adora1*$^{-/-}$, *Adora3*$^{-/-}$) mice. Confirming prior data, stimulation of the A_3 adenosine receptor (AR) induced hypothermia via peripheral mast cell degranulation, histamine release, and activation of central histamine H_1 receptors. In contrast, A_1AR agonists and AMP both acted centrally to cause hypothermia. Commonly used, selective A_1AR agonists, including N^6-cyclopentyladenosine (CPA), N^6-cyclohexyladenosine (CHA), and MRS5474, caused hypothermia via both A_1AR and A_3AR when given intraperitoneally. Intracerebroventricular dosing, low peripheral doses of Cl-ENBA [(±)-5'-chloro-5'-deoxy-N^6-*endo*-norbornyladenosine], or using *Adora3*$^{-/-}$ mice allowed selective stimulation of A_1AR. AMP-stimulated hypothermia can occur independently of A_1AR, A_3AR, and mast cells. A_1AR and A_3AR agonists and AMP cause regulated hypothermia that was characterized by a drop in total energy expenditure, physical inactivity, and preference for cooler environmental temperatures, indicating a reduced body temperature set point. Neither A_1AR nor A_3AR was required for fasting-induced torpor. A_1AR and A_3AR agonists and AMP trigger regulated hypothermia via three distinct mechanisms.

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Abbreviations: i.p., intraperitoneal; i.c.v., intracerebroventricular; AxAR, adenosine x receptor.

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1. Introduction

Mammals are endotherms, typically maintaining a warm core body temperature (Tb) of ~35 °C to 37 °C, depending on circadian fluctuations (Refinetti, 2010). Some small mammals, including mice, when exposed to an inadequate food supply in a quiet, cool environment use torpor to achieve significant energy conservation (Geiser, 2004; Melvin and Andrews, 2009). Torpor is an example of a regulated hypothermia (anapyrexia), which is characterized by a reduced Tb set point, metabolic rate, and physical activity, with Tb falling close to the environmental temperature. In regulated hypothermia multiple physiologic mechanisms are coordinated to cool the body, including vasodilation, decreased physical activity, reduced brown adipose tissue thermogenesis, and seeking a cool environment (Lute et al., 2014). Regulated hypothermia differs from hypothermia caused by cold exposure, where the body attempts, but is unable, to maintain Tb.

Hypothermia can also be caused by a wide variety of drugs and neurotransmitters (Clark and Lipton, 1985), although in most cases it is not documented if there is a reduction in Tb set point. Clinically, hypothermia is routinely employed to minimize tissue damage after hypoxic or ischemic injury (Arrich et al., 2012; Azzopardi et al., 2014). The hypothermia is typically induced with surface cooling. However, pharmacological reduction of the Tb might minimize undesired compensatory mechanisms (e.g., sympathetic activation, shivering). Development of a drug regimen that produces controlled, regulated hypothermia and avoids activating counter-regulatory responses would likely be of clinical utility (Drew et al., 2015; Tupone et al., 2014).

The hypothermic effect of adenosine was first reported in 1931 (Bennet and Drury, 1931). Once multiple adenosine receptor (AR) subtypes were identified, the hypothermia was attributed to action at A_1AR, likely within the brain (Anderson et al., 1994). Peripheral dosing of N^6-cyclohexyladenosine (CHA; see Fig. S1 for structures) elicited hypothermia, which was lost in A_1AR knock out (*Adora1*$^{-/-}$) mice (Johansson et al., 2001). CHA also caused hypothermia when infused directly into the nucleus of the solitary tract in rats (Tupone et al., 2013). However, some data suggested a contribution from non-A_1AR mechanisms, as only partial attenuation of hypothermia caused by N^6-R-phenylisopropyladenosine (R-PIA) was seen in *Adora1*$^{-/-}$ mice (Yang et al., 2007).

More recently, it has become clear that adenosine agonists also induce hypothermia in mice via A_3AR. A critical observation was that hypothermia caused by R-PIA was attenuated in A_3AR knock out (*Adora3*$^{-/-}$) mice (Yang et al., 2010). In rodents, A_3AR is expressed on immune cells (Borea et al., 2015) and A_3AR agonists trigger mast cell degranulation (Auchampach et al., 1997; Fozard et al., 1996; Salvatore et al., 2000). We have recently shown that A_3AR agonists activate peripheral mast cells releasing histamine, which then acts on central histamine H_1 receptors to lower the Tb set point (Carlin et al., 2016).

Adenosine 5′-monophosphate (AMP) is a proposed natural regulator of torpor and injection of a large dose of AMP (500–3500 mg/kg, i. p.) causes hypothermia (Zhang et al., 2006). Given the dose size, one might consider if some of the effects of AMP are due to its conversion to free adenosine (Rittiner et al., 2012; Swoap et al., 2007). The observation that AMP's hypothermic effects remain intact in mice lacking any one of the four AR subtypes, indicates that AMP is not acting non-redundantly via a single AR (Daniels et al., 2010). However, AMP-induced hypothermia was reported to be blocked by infusion of an A_1AR antagonist in the pre-optic area (Muzzi et al., 2013). The mechanism by which AMP causes hypothermia is currently unclear.

While studying hypothermia caused by A_3AR agonists, we observed that some nucleoside derivatives commonly used as A_1AR agonists also had activity at the A_3AR. This prompted the current re-examination and comparison of A_1AR agonists, A_3AR agonists, and AMP. Our data suggest that each of these can trigger hypothermia in mice via different mechanisms.

2. Materials and methods

2.1. Mice

Male C57BL/6J and *Kit*$^{W-sh/W-sh}$ mice (Stock #012861) (Grimbaldeston et al., 2005; Nigrovic et al., 2008) were obtained from the Jackson Laboratory. *Adora1*$^{-/-}$ mice on a C57BL/6J background were provided by Dr. Jurgen Schnermann (Sun et al., 2001) and genotyped by PCR (*Adora1* reverse common primer 5′-ACATGGGGGTTGAACAGAGA, *Adora1* forward primer 5′-AGCTGGCTACCGCTACACAT, and Neo forward primer 5′-TCTGGATTCATCGACTGTGG), producing 302 bp wild type and ~900 bp null allele products. *Adora3*$^{-/-}$ mice on a C57BL/6 background made by Merck (Salvatore et al., 2000) were provided by Dr. Stephen Tilley and genotyped by PCR (*Adora3* reverse common primer 5′- ACTGGCCCATACACAACCTG, *Adora3* forward primer 5′-AGACAATGAAATAGACGGTGGTG, and Neo forward primer 5′-ATGGAAGGATTGGAGCTACG), producing 208 bp wild type and ~400 bp null allele products. Mice were singly housed at ~22 °C with a 12:12-h light-dark cycle. Chow (NIH-07, Envigo Inc, Madison, WI) and water were available ad libitum. Mice were studied ≥7 days after any operation or prior treatment. Reuse of mice tends to reduce physical activity levels, presumably due to acclimatization. No specific effort was made to acclimatize mice to handling in individual experiments. Studies were approved by the Animal Care and Use Committee of National Institute of Diabetes and Digestive and Kidney Diseases.

2.2. Drugs

The following compounds (vehicle) were purchased from Sigma (St. Louis, MO) or Tocris (Minneapolis, MN): Cl-ENBA, (±)-5'-chloro-5'-deoxy-N^6-*endo*-norbornyladenosine (Franchetti et al., 2009; Trivedi et al., 1989) (10% DMSO in saline); CHA, N^6-cyclohexyladenosine (dissolved in DMSO, then diluted to 10% DMSO with saline); CPA, N^6-cyclopentyladenosine (saline); pyrilamine (saline); AMP, adenosine 5'-monophosphate (saline); CCPA, 2-chloro-N^6-cyclopentyladenosine. MRS5474, (1R,2R,3S,5S)-4-(2-chloro-6-((dicyclopropylmethyl)amino)-9H-purin-9-yl)bicyclo [3.1.0]hexane-2,3-diol, (dissolved in DMSO, then diluted with 9 vol 30% PEG400) was synthesized as described (Tosh et al., 2012b).

2.3. Adenosine receptor binding affinities

Binding affinity for mouse A_1AR, A_{2A}AR, and A_3ARs was measured as described (Kreckler et al., 2006) using membranes from human embryonic kidney (HEK)-293 cells stably expressing individual recombinant mouse adenosine receptors and using the agonists [^{125}I]N^6-(4-amino-3-iodobenzyl)adenosine-5'-methyluronamide ([^{125}I]AB-MECA; A_1AR and A_3AR) and [^{3}H]CGS21680 (A_{2A}AR) as radioligands. Nonspecific binding was defined using 100 μM adenosine-5′–*N*-ethylcarboxamide (NECA). K_i values were obtained using the Cheng-Prusoff equation from IC_{50} values calculated by non-linear regression analysis of specific binding data using GraphPad Prism software (San Diego, CA).

2.4. Central infusions

Mice were anesthetized with ketamine/xylazine (80/10 mg/kg, i.p.). Sterile guide cannulas (5.25 mm, 26 gauge, Plastics One,