Neuropharmacology xxx (2018) 1–11

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Activation of adenosine A_{2A} or A_{2B} receptors causes hypothermia in mice

Jesse Lea Carlin [a, 1], Shalini Jain [b, 1], Romain Duroux [c], R. Rama Suresh [c], Cuiying Xiao [a], John A. Auchampach [d], Kenneth A. Jacobson [c], Oksana Gavrilova [b, 2], Marc L. Reitman [a, *, 2]

[a] Diabetes, Endocrinology, and Obesity Branch, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892, USA
[b] Mouse Metabolism Core, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892, USA
[c] Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892, USA
[d] Department of Pharmacology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA

ARTICLE INFO

Article history:
Received 24 October 2017
Received in revised form
26 January 2018
Accepted 28 February 2018
Available online xxx

Keywords:
Hypothermia
Adenosine
A_{2A}AR
A_{2B}AR
Preoptic area
Paraventricular hypothalamus

ABSTRACT

Extracellular adenosine is a danger/injury signal that initiates protective physiology, such as hypothermia. Adenosine has been shown to trigger hypothermia via agonism at A_1 and A_3 adenosine receptors (A_1AR, A_3AR). Here, we find that adenosine continues to elicit hypothermia in mice null for A_1AR and A_3AR and investigated the effect of agonism at A_{2A}AR or A_{2B}AR. The poorly brain penetrant A_{2A}AR agonists CGS-21680 and PSB-0777 caused hypothermia, which was not seen in mice lacking A_{2A}AR. MRS7352, a likely non-brain penetrant A_{2A}AR antagonist, inhibited PSB-0777 hypothermia. While vasodilation is probably a contributory mechanism, A_{2A}AR agonism also caused hypometabolism, indicating that vasodilation is not the sole mechanism. The A_{2B}AR agonist BAY60-6583 elicited hypothermia, which was lost in mice null for A_{2B}AR. Low intracerebroventricular doses of BAY60-6583 also caused hypothermia, indicating a brain site of action, with neuronal activation in the preoptic area and paraventricular nucleus of the hypothalamus. Thus, agonism at any one of the canonical adenosine receptors, A_1AR, A_{2A}AR, A_{2B}AR, or A_3AR, can cause hypothermia. This four-fold redundancy in adenosine-mediated initiation of hypothermia may reflect the centrality of hypothermia as a protective response.

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1. Introduction

Maintenance of a warm, regulated core body temperature (Tb) is a defining feature of mammals and birds. In homeotherms, hypothermia can be caused by excessive cold exposure, severe injury or illness, and many drugs. In large mammals, such as adult humans, hypothermia is relatively difficult to achieve. In contrast, small mammals, such as mice, are at risk of hypothermia and expend relatively massive amounts of energy to maintain their Tb, a process in which brown fat has a large role. The high energetic cost of defending Tb means that small mammals have evolved strategies for using Tb reduction to conserve fuel. For example, with cold exposure or fasting, mice let their Tb fall slightly (~1 °C). Mice can also enter torpor, during which Tb can fall >10 °C, producing huge energetic savings (Gavrilova et al., 1999; Geiser, 2004; Hudson and Scott, 1979; Melvin and Andrews, 2009).

Hypothermia is observed with severe injury (Shafi et al., 2005) and sepsis (Fonseca et al., 2016). It is unknown if the poor prognosis associated with hypothermia is because hypothermia is a marker of clinical severity or if the hypothermia represents a physiologic response to the dire situation (Fonseca et al., 2016). Clinically, therapeutic hypothermia is used after insults such as neonatal hypoxic injury (Azzopardi et al., 2014) and cardiac arrest (Callaway et al., 2015), and prophylactically during certain types of hypoperfusion surgery (Yan et al., 2013). Therapeutic hypothermia is induced by cooling, sometimes with pharmacologic agents to block shivering. Drugs that induce hypothermia through reduction of the central Tb balance point could minimize counter-regulatory

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

* Corresponding author.
E-mail addresses: oksanag@mail.nih.gov (O. Gavrilova), marc.reitman@nih.gov (M.L. Reitman).

[1] Co-first authors.
[2] Co-senior authors.

https://doi.org/10.1016/j.neuropharm.2018.02.035
0028-3908/Published by Elsevier Ltd.

mechanisms such as shivering and provide clinical benefit.

Extracellular adenosine is one of the body's danger signals, an indicator of tissue damage or metabolic stress (Borea et al., 2016). Adenosine acts at four G protein-coupled receptors: A_1AR, A_{2A}AR, A_{2B}AR, and A_3AR. A_1AR and A_3AR are typically coupled to Gi, while A_{2A}AR and A_{2B}AR are usually coupled to Gs or Gq. Adenosine has many actions that dampen immune and inflammatory responses, one of which is causing hypothermia (Bennet and Drury, 1931). The best studied mechanism of adenosine-induced hypothermia is via agonism of brain A_1AR, with proposed sites of action being the anterior hypothalamus and the nucleus of the solitary tract (Anderson et al., 1994; Carlin et al., 2017; Johansson et al., 2001; Muzzi et al., 2013; Shintani et al., 2005; Tupone et al., 2013). However, mouse genetic and pharmacologic evidence suggested that adenosine can cause hypothermia via other mechanisms (Yang et al., 2007, 2010). Hypothermia via A_3AR is caused by agonism of peripheral mast cell A_3AR, causing histamine release, which produces hypothermia via central histamine H_1 receptors (Carlin et al., 2016). Since A_1AR agonists must penetrate the brain to produce hypothermia, some compounds used as A_1AR agonists may be causing hypothermia via peripheral mast cell A_3AR (Carlin et al., 2017).

There is limited information on the role of A_{2A}AR and A_{2B}AR in hypothermia. The A_{2A}AR agonist CGS-21680 produced mild hypothermia with a high i.c.v. dose (Anderson et al., 1994) or with peripheral dosing (Eisner et al., 2017). These effects were smaller than those seen with agonism at other adenosine receptors and were not investigated mechanistically. Intraarterial CGS-21680 caused hypotension in wild type but not *Adora2a*$^{-/-}$ mice, which may have been accompanied by hypothermia, but Tb was not measured (Ledent et al., 1997). In contrast, the A_{2A}AR agonist PSB-0777 increased energy expenditure by stimulation of brown adipose tissue; effects on Tb were not reported (Gnad et al., 2014). No role for A_{2B}AR in hypothermia has been identified (Fredholm et al., 2011).

Here we explored whether agonism at A_1AR and A_3AR completely accounts for the hypothermia potential of adenosine, or if A_{2A}AR and A_{2B}AR can also contribute. We find that agonism of either A_{2A}AR or A_{2B}AR does cause hypothermia, and investigate the pharmacology and mechanisms.

2. Materials and methods

2.1. Mice

Male C57BL/6 J and *Kit*$^{W-sh/W-sh}$ (JAX #012861) (Grimbaldeston et al., 2005; Nigrovic et al., 2008) mice were obtained from the Jackson Laboratory. *Adora1*$^{-/-}$ (Sun et al., 2001), *Adora3*$^{-/-}$ (Salvatore et al., 2000), and *Adora1*$^{-/-}$;*Adora3*$^{-/-}$ mice were obtained and genotyped as reported (Carlin et al., 2017). *Adora2a*$^{-/-}$ mice (Chen et al., 1999) on a mixed background were obtained from Dr. Dorian McGavern and genotyped as described (JAX #010685). *Adora2b*$^{-/-}$ mice (Hua et al., 2007) on a C57BL/6 J background were obtained from Dr. Stephen Tilley and genotyped as described (JAX #022499). Mice were singly housed at ~22 °C with a 12:12-h light-dark cycle (lights on at 6:00 a.m.). Chow (NIH-07, Envigo Inc, Madison, WI) and water were available ad libitum. Drugs were typically dosed at 10:00 a.m. 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

MRS7352 (4-((4-(3-(5-amino-2-(furan-2-yl)-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)propyl)phenoxy)methyl) benzenesulfonate triethylammonium salt) was synthesized and purified by HPLC as described (Duroux et al., 2017). The following compounds (vehicle) were purchased from Tocris (Minneapolis, MN): CGS-21680 (15% DMSO/15% KolliphorEL/70% saline for i. p.; 5% DMSO/95% PBS neutralized with one equivalent of NaOH for i.c.v.); PSB-0777 (saline); BAY60-6583 (25% PEG); SCH442416 (15% DMSO/15% KolliphorEL/70% saline). Adenosine (10% DMSO in water) was obtained from Sigma. APEC (2-[[2-[4-[2-(2-aminoethyl)-aminocarbonyl]ethyl]phenyl]ethylamino]-5′-N-ethyl-carboxamidoadenosine; water) was obtained from the NIMH Chemical Synthesis and Drug Supply Program, http://nimh-repository.rti.org/. Regadenoson was purchased from Santa Cruz Biotechnology, Inc. Screening of PSB-0777, SCH442416, BAY60-6583, and CGS-21680 for off target activities at 45 human receptors, channels, and transporters with radioligand binding assays was performed by the National Institute of Mental Health's Psychoactive Drug Screening Program (PDSP) directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill.

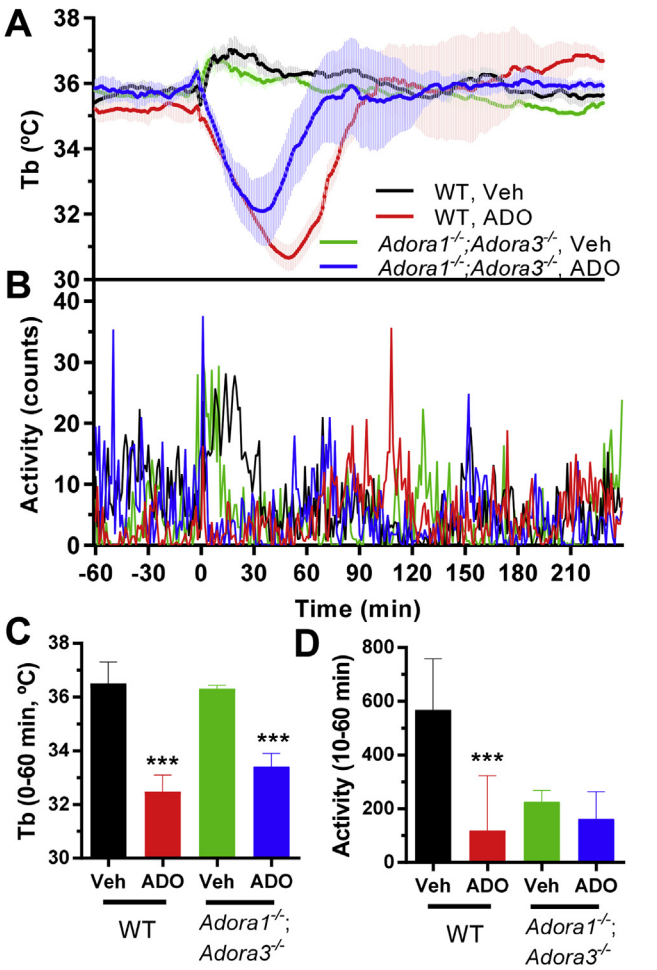

Fig. 1. Adenosine-mediated hypothermia remains intact in *Adora1*$^{-/-}$;*Adora3*$^{-/-}$ mice. (A,B) Tb and activity response to adenosine (ADO, 100 mg/kg, i. p.) or vehicle (Veh) in C57BL/6 J (WT) and *Adora1*$^{-/-}$;*Adora3*$^{-/-}$ mice. (C,D) Average Tb (0–60 min) and total physical activity (10–60 min). Data are mean ± SEM, n = 5–6/group in a crossover design; ***p < 0.001 vs vehicle within genotype.

Please cite this article in press as: Carlin, J.L., et al., Activation of adenosine A_{2A} or A_{2B} receptors causes hypothermia in mice, Neuropharmacology (2018), https://doi.org/10.1016/j.neuropharm.2018.02.035