Pharmacology, Biochemistry and Behavior 140 (2016) 82–89

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Administration of caffeine inhibited adenosine receptor agonist-induced decreases in motor performance, thermoregulation, and brain neurotransmitter release in exercising rats

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ARTICLE INFO

Article history:
Received 23 April 2015
Received in revised form 26 October 2015
Accepted 27 October 2015
Available online 18 November 2015

Keywords:
Caffeine
Core temperature
Dopamine
Ergogenic effect
Adenosine receptor

ABSTRACT

We examined the effects of an adenosine receptor agonist on caffeine-induced changes in thermoregulation, neurotransmitter release in the preoptic area and anterior hypothalamus, and endurance exercise performance in rats. One hour before the start of exercise, rats were intraperitoneally injected with either saline alone (SAL), 10 mg kg^{-1} caffeine and saline (CAF), a non-selective adenosine receptor agonist (5′-N-ethylcarboxamidoadenosine [NECA]: 0.5 mg kg^{-1}) and saline (NECA), or the combination of caffeine and NECA (CAF + NECA). Rats ran until fatigue on the treadmill with a 5% grade at a speed of 18 m min^{-1} at 23 °C. Compared to the SAL group, the run time to fatigue (RTTF) was significantly increased by 52% following caffeine administration and significantly decreased by 65% following NECA injection (SAL: 91 ± 14.1 min; CAF: 137 ± 25.8 min; NECA: 31 ± 13.7 min; CAF + NECA: 85 ± 11.8 min; $p < 0.05$). NECA decreased the core body temperature (T_{core}), oxygen consumption, which is an index of heat production, tail skin temperature, which is an index of heat loss, and extracellular dopamine (DA) release at rest and during exercise. Furthermore, caffeine injection inhibited the NECA-induced decreases in the RTTF, T_{core}, heat production, heat loss, and extracellular DA release. Neither caffeine nor NECA affected extracellular noradrenaline or serotonin release. These results support the findings of previous studies showing improved endurance performance and overrides in body limitations after caffeine administration, and imply that the ergogenic effects of caffeine may be associated with the adenosine receptor blockade-induced increases in brain DA release.

1. Introduction

Caffeine is widely consumed as an ergogenic aid to improve cognitive and physical performance during exercise and military operations. Endurance performance can be improved when humans are administrated approximately 3 to 13 mg kg^{-1} caffeine (Sökmen et al., 2008). Davis et al. (2003) reported that intracerebroventricular administration of 200 μg of the non-selective adenosine receptor agonist 5′-N-ethylcarboxamidoadenosine (NECA) inhibited the run time to fatigue (RTTF), while central nervous system (CNS) administration of 200 μg of caffeine inhibited the NECA-induced decreases in endurance exercise performance. These results indicate that the ergogenic effect of caffeine may involve the blockage of adenosine receptors in the brain. However, the precise mechanism behind these effects remains elusive. Moreover, adenosine has been shown to be an inhibitory modulator of neuronal excitability and synaptic transmission in the brain via the activation of adenosine receptors. Several studies suggested that adenosine inhibited the release of most brain excitatory neurotransmitters (Harms et al., 1987; Okada et al., 1997), especially dopamine (DA; Fredholm et al., 1999; Myers and Pugsley, 1986). Therefore, the ergogenic effect of caffeine may be due to the antagonism of adenosine receptors, thus affecting neurotransmitter release. However, currently, there is no evidence to support this notion.

DA is an excitatory neurotransmitter. Depletion or reduction of central DA levels is associated with central fatigue and reduction of endurance exercise performance (Davis and Bailey, 1997). Previous studies demonstrated that the ergogenic effects of DA affect arousal, emotion, motivation, and motor control. Recently, some researchers have suggested that the DA-induced improvements in exercise performance may occur by affecting other physiological responses, such as by inhibiting hyperthermia-induced fatigue (Roelands and Meeusen, 2010; Watson et al., 2005). Several studies have provided evidence that the activation of dopaminergic neurotransmission in the preoptic area and anterior hypothalamus (PO/AH), which is an important locus for thermoregulation during exercise, may improve heat tolerance and inhibit hyperthermia-induced fatigue during exercise (Balthazar et al., 2009, 2010; Hasegawa et al., 2008).

Adenosine has been shown to affect thermoregulation (Matuszek and Gagalo, 1996; Zarrindast and Heidari, 1993). Central administration of adenosine caused dose-related hypothermia in mice (Anderson et al., 1994). It has been reported that the increase in brain temperature activates adenosine receptors in brain slices,

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http://dx.doi.org/10.1016/j.pbb.2015.10.019
0091-3057/

markedly inhibiting excitatory synaptic transmission (Masino and Dunwiddie, 1999; Masino et al., 2001). These previous studies also suggested that the activation of adenosine receptors inhibited the increase in core body temperature (T_{core}) and prevented potential hyperthermia-induced tissue damage. As hyperthermia has been described as an important factor that can lead to central fatigue via a reduction in the CNS drive for exercise, hyperthermia-impaired exercise performance may be related to adenosine receptors. The activation of adenosine receptors may inhibit excitatory synaptic transmission, decrease extracellular DA release, impair the heat tolerance during exercise, stimulate inhibitory signals arising from the CNS to cease exercise, and inhibit exercise induced-hyperthermia. If the mechanism that hyperthermia-impaired exercise performance may be related to the activation of adenosine receptors is reliable, then the ergogenic effect of caffeine is likely due to blockage of the adenosine receptors, which inhibits hyperthermia induced-fatigue. However, this mechanism needs further study.

Recently, we reported that intraperitoneal administration of 10 mg kg^{-1} caffeine improved endurance exercise performance, increased exercise-induced hyperthermia, and stimulated extracellular DA release in the PO/AH in exercising rats (Zheng et al., 2014). Furthermore, caffeine was able to override the critical limiting T_{core} during exercise. These results indicate that caffeine has ergogenic and hyperthermic effects, and imply that these effects may be associated with changes in DA release in the brain. Although the direct release of intracellular calcium and the inhibition of cyclic nucleotide phosphodiesterases have been cited as the biochemical mechanisms of caffeine, the dose that intraperitoneally injected 10 mg kg^{-1} caffeine in the rat may be too low to induce these actions of caffeine in the brain (Fredholm et al., 1999). Therefore, caffeine-induced increases in exercise performance and extracellular DA release may be associated with the blockage of adenosine receptors. However, there is currently no evidence to support this hypothesis. The purpose of the present study was to examine the effects of an adenosine receptor agonist on caffeine-induced changes in thermoregulation, neurotransmitter release in the PO/AH, and endurance exercise performance in rats. We hypothesized that the administration of caffeine would inhibit adenosine receptor agonist-induced decreases in performance, T_{core}, heat production responses, and extracellular DA release in the PO/AH during exercise.

2. Materials and methods

2.1. Animals

Male Wistar rats (Shimizu jiken, Shizuoka, Japan, weighing 300–350 g) were used in all experiments. Animals were housed in a room of normal ambient temperature (23 ± 1 °C), on a 12 h light/dark cycle (lights on at 06:00 h). Animals had a standard diet with free access to food and water throughout the experiments. All experiments were approved by the Ethical Committee for Animal Experiments of Hiroshima University.

2.2. Surgeries and exercise familiarization sessions

A telemetry device (TA10TA-F40, Data Science International, MN, USA) was implanted into the peritoneal cavity under pentobarbital anesthesia (50 mg kg^{-1}, I.P.), then the intracerebral guide cannula (CXG-12, Eicom, Kyoto, Japan) was implanted in the left lateral PO/AH (anterior −0.3 mm, lateral +0.8 mm, ventral −6.7 mm, relative to bregma) on the same day. One week after surgery, successfully recuperated rats were exercised for 5 days on a rodent treadmill. Each daily session consisted of running for 5 min with a 5% grade at a speed of 18 m min^{-1} (Cordeiro et al., 2014).

2.3. Experimental procedures

On the day of the experiments, rats were anesthetized with isoflurane 4% and oxygen insufflated into a transparent chamber. After induction, the dummy cannula was replaced by a microdialysis probe with a membrane length of 2 mm (CX-I-12-02, Eicom). The guide cannula with the probe was connected by the parafilm. The microdialysis probe was connected to a microinjection pump (CMA 100, CMA Microdialysis, Stockholm, Sweden) and was perfused with a modified Ringer's solution (147 mM NaCl, 4 mM KCl and 2.3 mM $CaCl_2$) at a flow rate of 2 μl min^{-1}. The air-tight treadmill chamber (MK-680AT/02R, Muromachi Kikai, Tokyo, Japan) was adjusted by attaching the counter balance arm of the microdialysis system (Hasegawa et al., 2011). Tail skin temperature (T_{tail}), which is an index of heat loss responses (Hasegawa et al., 2011), was measured on the dorsal surface of the skin about 10 mm from the base of the tail using an alumel-chromel thermocouple (KT-GSHV 1P-1/0.32 mm, Takeda special electric wire works, Kobe, Japan). The thermocouple was attached with tape. On the day of the experiment, baseline collections on the treadmill were obtained for 1 h. One hour before the start of exercise, rats were intraperitoneally injected with either saline (SAL), 10 mg kg^{-1} caffeine (CAF), a non-selected adenosine receptor agonist (NECA: 0.5 mg kg^{-1}) or the combination of caffeine and NECA. Rats ran until fatigue on the treadmill with a 5% grade at a speed of 18 m min^{-1}at 23 °C. Fatigue was considered to have occurred when the rat was unable to keep pace with the treadmill and stayed on the grid positioned at the back of the treadmill for a period of 20 s. We continued to monitor for another two hours during the recovery period after treadmill running. We set out four experimental conditions: the injection of saline only (SAL), the injection of caffeine and saline (CAF), the injection of adenosine agonist NECA and saline (NECA) and the injection of caffeine and NECA (CAF + NECA). The treatment order was randomized and used in a double-blind crossover manner. All rats completed all four experimental conditions, which were separated by 5 days to ensure drug washout period.

2.4. Drugs

Caffeine hydrate (Kenei Drug Company, Osaka, Japan) was dissolved in saline. NECA (Sigma Aldrich, Tokyo, Japan) was also dissolved in saline. Freshly prepared drugs were administered intraperitoneally (1 ml kg^{-1}) in all experiments. According to Myers and Pugsley (1986), we used 0.5 mg kg^{-1} NECA in the present study.

2.5. Measurement of oxygen consumption

The gas analysis system consisted of two air-tight treadmill chambers. Oxygen consumption (VO_2), which is an index of heat production (Hasegawa et al., 2011), was continuously measured with an O_2/CO_2 metabolism measuring system (MK-5000RQ/02; Muromachi Kikai). Room air was pumped through the chambers at a rate of 3.0 l min^{-1}. VO_2 was collected every 3 min. We excluded the data for 6 min after intraperitoneal injection because the chamber door was opened.

2.6. Calculations

Body heat rate (BHR; °C min^{-1}) was calculated as BHR = ΔT_{core}/running time interval, where ΔT_{core} represents the change in T_{core} (T_f-T_i) and T_f and T_i represent T_{core} at the fatigue point and prior to exercise, respectively. Heat storage was calculated as HS = $\Delta T_{core} \cdot m \cdot c$, where m represents body weight in grams and c represents specific heat of the body tissues (0.826 cal g^{-1} °C^{-1}).