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Acute intraperitoneal injection of caffeine improves endurance exercise performance in association with increasing brain dopamine release during exercise

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ABSTRACT

The purpose of this study was to examine changes of thermoregulation, neurotransmitters in the preoptic area and anterior hypothalamus (PO/AH), which is the thermoregulatory center, and endurance exercise performance after the intraperitoneal injection of caffeine in rats. Core body temperature (T_{core}), oxygen consumption (VO_2) and tail skin temperature (T_{tail}) were measured. A microdialysis probe was inserted in the PO/AH, and samples for the measurements of extracellular dopamine (DA), noradrenaline (NA) and serotonin (5-HT) levels were collected. During the rest experiment, 1 h after baseline collections in the chamber (23 °C), the rats were intraperitoneally injected with saline, or 3 mg kg^{-1} or 10 mg kg^{-1} caffeine. The duration of the test was 4 h. During the exercise experiment, baseline collections on the treadmill were obtained for 1 h. One hour before the start of exercise, rats were intraperitoneally injected with either 10 mg kg^{-1} caffeine (CAF) or saline (SAL). Animals ran until fatigue at a speed of 18 m min^{-1}, at a 5% grade, on the treadmill in a normal environment (23 °C). At rest, 3 mg kg^{-1} caffeine did not influence T_{core}, T_{tail}, VO_2, extracellular DA, NA and 5-HT. 10 mg kg^{-1} caffeine caused significant increases in T_{core}, VO_2, T_{tail} and extracellular DA in the PO/AH. In addition, 10 mg kg^{-1} caffeine increased the run time to fatigue (SAL: 104.4 ± 30.9 min, CAF: 134.0 ± 31.1 min, $p < 0.05$). The combination of caffeine and exercise increased T_{core}, VO_2, T_{tail} and extracellular DA in the PO/AH. NA increased during exercise, while neither caffeine nor exercise changed 5-HT. These results indicate that caffeine has ergogenic and hyperthermic effects, and these effects may be related to changes of DA release in the brain.

1. Introduction

Caffeine is widely consumed as an ergogenic aid to improve cognitive and physical performance during exercise and military operations. From a review of previous studies, endurance performance can be improved when humans are administrated with approximately 3 to 13 mg kg^{-1} caffeine (Sökmen et al., 2008). Two animal studies have shown that the peripheral administration of caffeine at doses 4 and 6 mg kg^{-1} extends the run time to fatigue (Lim et al., 2001; Ryu et al., 2001). Several mechanisms have been proposed to explain caffeine's ergogenic effects, including increased myofibrillar calcium availability, enhanced exercise metabolism and substrate availability, and stimulation of the central nervous system (CNS) (Kalmar and Cafarelli, 2004). The administration of caffeine also enhanced cognition and inhibited muscle pain during exercise as a result of its effects on the CNS, but the precise mechanism in the CNS behind caffeine's ergogenic activity remains unclear.

Caffeine is a non-selective adenosine receptor antagonist. It can easily cross the blood–brain barrier, block adenosine receptors (especially adenosine A_1 and A_{2A} receptors), inhibit the effects of adenosine and influence neurotransmitter release (Fredholm et al., 1999). The administration of caffeine increased dopamine (DA) release in the striatum (Okada et al., 1996), the nucleus accumbens shell (NAc) (Solinas et al., 2002), and the caudate nucleus (Morgan and Vestal, 1989). Caffeine also influenced noradrenaline (NA) release (Whitham et al., 2006) and has the potential to reduce serotonin (5-HT) level during exercise (Lim et al., 2001). Moreover, DA influences some physiological responses and mechanisms that could similarly modify running performance, such as cognition, arousal, reward, motivation, sympathetic nervous system activities, as well as stress response and motor control (Balthazar et al., 2009). Animal studies have suggested that increased DA levels improves endurance exercise performance (Gerald, 1978), and reduced DA levels in the brain impairs the run time to exhaustion (Heyes et al., 1988). Therefore, blocking adenosine receptor-induced alterations in brain DA release may play an important role in the mechanism of caffeine's ergogenic effect. However, no studies have examined

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the direct effect of caffeine on brain neurotransmitter release during exercise.

Caffeine influences many physiological systems, including thermoregulation (Schlosberg, 1983). Acute administration of caffeine (<50 mg kg^{-1}) was shown to produce a dose-dependent increase in the core body temperature (T_{core}) of rats at rest (Pechlivanova et al., 2010; Schlosberg, 1983), but acute ingestion of caffeine in humans yielded conflicting results with respect to changes in thermoregulation during exercise. It has been reported that the preoptic area and anterior hypothalamus (PO/AH) may be the primary regions for body temperature regulation (Boulant, 2000). PO/AH inhibition by tetrodotoxin, which is used to block neurotransmission in specific brain regions, altered the increase in T_{core} of exercising rats by increasing heat production and decreasing heat loss. Therefore, it has been suggested that PO/AH is an important thermoregulatory site in the brain during exercise, and neurotransmitters in the PO/AH affect thermoregulation (Hasegawa et al., 2005a). Furthermore, DA is also involved in thermoregulation, particularly in the PO/AH during rest and exercise. Increased DA availability in the brain has a performance-enhancing effect, which is mediated by improvements in the tolerance to heat storage and increases in the metabolic rate induced by graded exercise (Balthazar et al., 2009). Recently, blockade of central dopamine D_1 and D_2 receptors was shown to impair exercise performance in rats by decreasing the tolerance to heat storage (Balthazar et al., 2010). As described above, given that caffeine can affect neurotransmitter release in the brain, the mechanism of caffeine's effect on thermoregulatory responses might be due to neurotransmitter alterations in the PO/AH.

Above all, the purpose of this study was to examine the changes in thermoregulation, neurotransmitter release in the PO/AH and endurance exercise performance after the administration of caffeine. In addition, to determine the dose of caffeine in the exercise experiment and to exclude the effect of exercise on extracellular DA release in the brain, we first examined the effect of different doses of caffeine on thermoregulatory responses and neurotransmitter release in the PO/AH at rest. We hypothesized that the administration of caffeine would improve exercise performance, affect thermoregulatory responses and increase extracellular DA release in the PO/AH.

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, 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 at a speed of 18 m min^{-1}, at a 5% grade (Cordeiro et al., 2014).

2.3. Experimental procedures

Each rat performed three experimental conditions. 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 counterbalance arm of the microdialysis system (Hasegawa et al., 2011). Tail skin temperature (T_{tail}) 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. During the rest experiment, 1 h after baseline collections in the chamber (23 °C), the rats were intraperitoneally injected with saline (CAF0: 1 ml kg^{-1} of saline without caffeine), or 3 mg kg^{-1} (CAF3) or 10 mg kg^{-1} (CAF10) caffeine. The duration of the test was 4 h. For the exercise experiment, each rat performed two experimental conditions. During the exercise experiment, baseline collections on the treadmill were obtained for 1 h. One hour before the start of exercise, rats were intraperitoneally injected with either 10 mg kg^{-1} caffeine (CAF) or saline (SAL). Animals ran until fatigue at a speed of 18 m min^{-1}, at a 5% grade, on the treadmill in a normal environment (23 °C). Exhaustion 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 30 s (Davis et al., 2003). We continued to monitor for another 2 h during the recovery period after treadmill running. The treatment order was randomized and used in a double-blind crossover manner. Furthermore, all rats completed all experimental conditions, which were separated by 5 days to ensure drug washout period.

2.4. Drugs

Caffeine and saline were used in the study. Caffeine hydrate (Kenei Drug Company, Osaka, Japan) was dissolved directly with saline. Freshly prepared drugs were administered intraperitoneally in all experiments. It is reported that the absorption of caffeine is complete within approximately 1 h after ingestion (typically 99% of the ingested dose is absorbed within 45 min), and seems to be dose-independent, at least for doses usually consumed, i.e., up to 10 mg kg^{-1} (Magkos and Kavouras, 2005). In addition, pharmacokinetics is comparable after oral or intraperitoneal administration of caffeine in humans and animals, leading to superimposable plasma curves (Fredholm et al., 1999). Therefore, to promote the caffeine levels during exercise, rats were injected with caffeine before 60 min of the start of exercise.

2.5. Measurement of oxygen consumption

The gas analysis system consisted of two air-tight treadmill chambers (as described above). Oxygen consumption (VO_2) 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} (Hasegawa et al., 2011). 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. Sampling

During the experiment, T_{core} was measured and monitored by a biotelemetry system (Dataquest A.R.T., Data Science International). T_{tail}, which is an index of heat loss, and VO_2, which is an index of heat production, were also simultaneously measured. Microdialysis samples (20 μl) were collected every 10 min. These parameters were recorded at rest, during treadmill exercise, and during the 120 min of recovery.