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Fasting activated histaminergic neurons and enhanced arousal effect of caffeine in mice

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ABSTRACT

Caffeine, a popular psychoactive compound, promotes wakefulness via blocking adenosine A_{2A} receptors in the shell of the nucleus accumbens, which projects to the arousal histaminergic tuberomammillary nucleus (TMN). The TMN controls several behaviors such as wakefulness and feeding. Fasting has been reported to activate the TMN histaminergic neurons to increase arousal. Therefore, we propose that caffeine may promote greater arousal under fasting rather than normal feeding conditions. In the current study, locomotor activity recording, electroencephalogram (EEG) and electromyogram recording and c-Fos expression were used in wild type (WT) and histamine H_1 receptor (H_1R) knockout (KO) mice to investigate the arousal effects of caffeine under fasting conditions. Caffeine (15 mg/kg) enhanced locomotor activity in fasted mice for 5 h, but only did so for 3 h in normally fed animals. Pretreatment with the H_1R antagonist pyrilamine abolished caffeine-induced stimulation on locomotor activity in fasted mice. EEG recordings confirmed that caffeine-induced wakefulness for 3 h in fed WT mice, and for 5 h in fasted ones. A stimulatory effect of caffeine was not observed in fasted H_1R KO mice. Furthermore, c-Fos expression was increased in the TMN under fasting conditions. These results indicate that caffeine had greater wakefulness-promoting effects in fasted mice through the mediation of H_1R.

1. Introduction

Caffeine, a constituent of coffee and other beverages, is a globally used psychoactive for arousal and diminution of fatigue. Caffeine is an antagonist at both adenosine A_1 (A_1Rs) and A_{2A} receptors ($A_{2A}Rs$) with similar affinities (Fredholm et al., 2011), and our previous studies using global A_1Rs and $A_{2A}Rs$ genetic knockout (KO) mice indicated that caffeine's promotion of wakefulness was due to $A_{2A}Rs$ blockade, but not A_1Rs (Huang et al., 2005). We used site-specific gene manipulations, including $A_{2A}R$ KO mice based on the Cre/loxP technology in mice and focal RNA interference, to silence $A_{2A}Rs$ expression in rats via local infection with adeno-associated virus (AAV) carrying short-hairpin RNA specific for the $A_{2A}Rs$ mRNA; the $A_{2A}Rs$ in the nucleus accumbens (NAc) shell are responsible for the wakefulness-promoting effect of caffeine (Lazarus et al., 2011). With AAV-encoding humanized Renilla green fluorescent protein to trace long axonal pathways, we found that $A_{2A}Rs$ in the NAc shell projected to the arousal nuclei, including histaminergic tuberomamillary nucleus (TMN) (Zhang et al., 2013). Thus, we suggest that histaminergic neurons in the TMN may be involved in the arousal effect of caffeine.

Histaminergic neurons primarily located in the TMN control several behaviors such as feeding and wakefulness (Panula and Nuutinen, 2013). Fasting has been reported to activate histaminergic neurons in the TMN and increase arousal, allowing goal-directed behavior such as obtaining food (Valdes et al., 2010). The TMN receives projections from orexin neurons in the lateral hypothalamic area (LH),

Abbreviations: $A_{2A}Rs$, adenosine A_{2A} receptors; AAV, adeno-associated virus; EEG, electroencephalogram; EMG, electromyogram; H_1R, histamine H_1 receptor; KO, knockout; LH, lateral hypothalamic area; NAc, nucleus accumbens; NREM, non-rapid eye movement; REM, rapid eye movement; TMN, tuberomammillary nucleus; WT, wild type.

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which coordinate sleep-wakefulness and motivated behaviors such as food seeking, especially during fasting stress (Sakurai, 2003). This region also has extensive projections to the arousal nucleus, such as the dorsal raphe, laterodorsal tegmentum and cholinergic basal forebrain regions (Brown et al., 2002; Panula et al., 1989; Valdes et al., 2010). These observations suggest that the histaminergic TMN is critical for increased arousal under fasting conditions. Therefore, we speculate that caffeine enhances this wake-promoting effect under fasting conditions via manipulating the histaminergic system.

H_1 receptors (H_1Rs) mediate arousal in the histaminergic system of the TMN (Lin et al., 1988; Monti et al., 1986; Tokunaga et al., 2009). Thus, we measured locomotor activity, recorded electroencephalogram (EEG) and electromyogram (EMG) and used immunohistochemical method in wild type (WT) and H_1R KO mice to explore the mechanisms behind caffeine-induced stimulation under fasting conditions. We observed that the histaminergic system is involved in enhancing caffeine's effect on wakefulness in fasted mice.

2. Materials and methods

2.1. Animals

Male SPF inbred C57BL/6J mice, weighing 20–26 g (11–13 weeks old), were obtained from the Laboratory Animal Center, Chinese Academy of Sciences (Shanghai, China). Male H_1R KO (Huang et al., 2006; Inoue et al., 1996) and their WT mice of the inbred C57BL/6 strain were generated from heterozygotes, weighing 20–26 g (11–13 weeks old), were maintained at Oriental Bioservice (Kyoto, Japan) and used in these experiments. Animals were housed at an ambient temperature of 22 ± 0.5°C at a relative humidity of 60 ± 2% and an automatically controlled 12 h light/12 h dark cycle (lights on at 07:00, illumination intensity ≈ 100 lux). Animals had *ad libitum* access to food and water. All experimental protocols were approved by the Animal Administrative Committee of Shanghai and Animal Care Committee of Osaka Bioscience Institute. Every effort was made to minimize the number of animals used and to minimize pain and discomfort experienced by the animals.

2.2. Fasting

Mice were kept as depicted above. Food was removed from the cages of experimental mice at dark onset at 19:00.

2.3. Locomotor activity recordings

Locomotor activity for an individual mouse was measured with a passive infrared sensor (Biotex, Kyoto, Japan) placed 17.5 cm above the floor of the recording cage (28 cm × 16.5 cm × 13 cm) as previously reported (Liu et al., 2012).

2.4. Polygraphic recordings and vigilance state analysis

Under pentobarbital anesthesia (50 mg/kg, i.p.), mice were implanted with electrodes for polysomnographic EEG and EMG recordings. Two stainless steels screws (1 mm in diameter) were inserted through the skull (antero-posterior, +1.0 mm; left-right, −1.5 mm from the bregma or lambda) according to the mouse brain atlas; these screws served as EEG electrodes. Two Teflon-coated, insulated stainless steel wires were placed bilaterally into both trapezius muscles, and these served as the EMG electrodes. All electrodes were attached to a microconnector and fixed onto the skull using dental cement.

After a 10-day recovery time, each mouse was transferred to a sound-proof recording chamber and connected to an EEG/EMG recording cable for a 4-day period of habituation to the experimental environment. Then the polygraphic recordings were recorded for 48 h in freely moving mice.

Cortical EEG and EMG signals were amplified, filtered (EEG, 0.5–30 Hz; EMG, 20–200 Hz) and then digitized at a sampling rate of 128 Hz and recorded by using SleepSign (Kissei Comtec, Nagano, Japan) as described earlier (Huang et al., 2005; Qu et al., 2010; Wang et al., 2012, 2015). When completed, polygraphic recordings were automatically scored off-line at 4-s epochs as wakefulness, rapid eye movement (REM) and non-rapid eye movement (NREM) sleep by SleepSign according to standard criteria. Finally, defined sleep-wake stages were examined visually, and corrected when necessary.

2.5. Pharmacological treatments

Caffeine (Wako, Osaka, Japan) and pyrilamine (Sigma-Aldrich, Saint Louis, MO, USA) were dissolved in sterile saline immediately before intraperitoneal (i.p.) administration to the mice at 09:00 or 08:00 on the experimental day at the dose of 15 mg/kg or 10 mg/kg. For baseline data, mice were given the same volume of vehicle at 09:00 or 08:00.

2.6. c-Fos immunohistochemistry

Four groups of mice were studied. Each group was given caffeine (15 mg/kg, i.p.), or vehicle at 09:00, respectively, under fed or fasted conditions. Next 5 h after caffeine or vehicle administration, animals were anesthetized with 10% chloral hydrate and perfused via the heart with saline solution followed by ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed, post-fixed in 4% paraformaldehyde for 6 h and then immersed in 30% sucrose overnight. Thereafter, frozen sections of brain were cut in coronal planes, at a thickness of 30 μm with a freezing microtome (Microm HM 525, Thermoscientific, Germany). Sections were stored in a cryo-protectant solution at 20°C for histological analysis.

Immunohistochemistry was performed on free-floating sections as described previously. In brief, sections were incubated with 0.3% hydrogen peroxide in 0.1 M phosphate-buffered saline (PBS, pH 7.4) for 15 min to quench endogenous peroxidase activity. After washing in PBS, sections were treated at room temperature in 3% normal donkey serum and 0.25% Triton X-100 in PBS (PBS-T) for 1 h, followed by primary rabbit anti-c-Fos (1:5000, CalBiochem, San Diego, CA, USA) antibody diluted in PBS-T with 0.02% sodium azide, overnight. After overnight incubation with the primary antisera, sections were rinsed and incubated for 2 h in biotinylated anti-rabbit secondary antiserum (Jackson ImmunoResearch, West Grove, PA, USA) at a dilution of 1:1000. All sections were then treated with avidin–biotin–peroxidase complex (1:1000, Vectastain ABC Elite kit, Vector Laboratories, Burlingame, CA, USA) for 1 h. The peroxidase reaction was visualized with 0.05% 3,3′-diaminobenzidine (Sigma-Aldrich, Saint Louis, MO, USA) in 0.1 M PB and 0.01% hydrogen peroxide. After terminating the reaction with PBS-azide, sections were mounted, dehydrated and covered. Adjacent sections were incubated as controls, without the primary antibody to confirm a lack of non-specific staining. Sections were examined under bright-field illumination using an Olympus BX51 microscope (Japan). Images were captured with a digital camera (DP72, Olympus, Japan).

2.7. Statistical analysis

All results are expressed as means ± SEM. For vigilance studies, amounts of sleep-wake states were expressed in minutes. The statistical significance of time course data for locomotor activity, sleep amount, stage transition, number of each stage bouts and mean duration was assessed with a two-tailed unpaired Student's *t*-test. Differences in drug treatment between fed and fasted mice were compared with a two-way ANOVA (with food and drug treatment as factors) followed by a *post hoc* Fisher's probable least-squares difference when appropriate. In all cases, $p < 0.05$ was the level of significance.