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Increase in cocaine- and amphetamine-regulated transcript (CART) in specific areas of the mouse brain by acute caffeine administration

Jin Hee Cho, Yun Ha Cho, Hyo Young Kim, Seung Ha Cha, Hyun Ryu, Wooyoung Jang, Kyung Ho Shin *

Department of Pharmacology, Korea University College of Medicine, Anam-Dong, Sungbuk-Gu, Seoul, Republic of Korea

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ABSTRACT

Caffeine produces a variety of behavioral effects including increased alertness, reduced food intake, anxiogenic effects, and dependence upon repeated exposure. Although many of the effects of caffeine are mediated by its ability to block adenosine receptors, it is possible that other neural substrates, such as cocaine- and amphetamine-regulated transcript (CART), may be involved in the effects of caffeine. Indeed, a recent study demonstrated that repeated caffeine administration increases CART in the mouse striatum. However, it is not clear whether acute caffeine administration alters CART in other areas of the brain. To explore this possibility, we investigated the dose- and time-dependent changes in CART immunoreactivity (CART-IR) after a single dose of caffeine in mice. We found that a high dose of caffeine (100 mg/kg) significantly increased CART-IR 2 h after administration in the nucleus accumbens shell (AcbSh), dorsal bed nucleus of the stria terminalis (dBNST), central nucleus of the amygdala (CeA), paraventricular hypothalamic nucleus (PVN), arcuate hypothalamic nucleus (Arc), and locus coeruleus (LC), and returned to control levels after 8 h. But this increase was not observed in other brain areas. In addition, caffeine administration at doses of 25 and 50 mg/kg appears to produce dose-dependent increases in CART-IR in these brain areas; however, the magnitude of increase in CART-IR observed at a dose of 50 mg/kg was similar or greater than that observed at a dose of 100 mg/kg. This result suggests that CART-IR in AcbSh, dBNST, CeA, PVN, Arc, and LC is selectively affected by caffeine administration.

1. Introduction

Caffeine is one of the most popular psychostimulants consumed worldwide (Barone and Roberts, 1996; Fredholm et al., 1999). Caffeine not only promotes alertness and arousal, but also sustains attention under conditions of sleep deprivation (Barry et al., 2005; Penetar et al., 1993; Wesensten et al., 2005). Caffeine also induces a slight anorectic effect (Racotta et al., 1994) and can produce anxiety and sleep alteration when consumed in high doses (Yanik et al., 1987). In addition, individuals who repeatedly consume caffeine have the potential to develop dependence and withdrawal symptoms (Strain et al., 1994). Although many of the effects of caffeine are likely associated with its ability to block adenosine receptors, additional mechanisms by which caffeine exerts its effects on brain function are only partially understood.

Among the potential mechanisms that may be involved in the effects of caffeine, cocaine- and amphetamine-regulated transcript (CART) is interesting due to its known involvement in drug dependence, anxiety, and regulation of food intake (Asakawa et al., 2001; Jaworski et al., 2003, 2008; Kim et al., 2003; Larsen and Hunter, 2006; Rogge et al., 2008; Subhedar et al., 2014). CART is distributed in the nucleus accumbens shell (AcbSh), dorsal bed nucleus of the stria terminalis (dBNST), central nucleus of the amygdala (CeA), paraventricular hypothalamic nucleus (PVN) and arcuate hypothalamic nucleus (Arc), locus coeruleus (LC), and other brain areas (Koylu et al., 1998). Repeated treatment with cocaine and amphetamine selectively increases CART level in the nucleus accumbens (Douglass et al., 1995), which appears to attenuate cocaine- and amphetamine-induced locomotor sensitization and self-administration (Jaworski et al., 2003, 2008; Kim et al., 2003; Peng et al., 2014). CART is also involved in anxiety-like behavior, as manifested by previous findings that microinjection of CART into the CeA or lateral ventricle reduces time spent in social interaction or in open arms in elevated plus maze (Chaki et al., 2003; Dandekar et al., 2008). Furthermore, CART in the hypothalamus appears to regulate food intake (Abbott et al., 2001). Given that CART is involved in drug dependence, anxiety, and regulation of food intake, it is probable that the regulation of CART in the brain may be involved in some of the effects of caffeine.

In support of this possibility, a recent study demonstrated that repeated caffeine administration increases CART in the striatum (Hu

* Corresponding author. Department of Pharmacology, Korea University College of Medicine, 126-1, 5-ga, Anam-dong, Sungbuk-gu, Seoul 136-705, Republic of Korea.
E-mail address: kyungho@korea.ac.kr (K.H. Shin).

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et al., 2014). However, the effect of acute caffeine administration on CART in other areas of the brain remains unclear. To assess the effects of acute caffeine administration on CART level in more detail, we investigated the time-dependent changes of CART immunoreactivity (CART-IR) in mouse brain after a single administration of high dose caffeine (100 mg/kg). We also evaluated dose-dependent changes in CART-IR 2 h after the administration of caffeine (25, 50, or 100 mg/kg) or saline. Two hours was chosen because 100 mg/kg caffeine administration produced a maximum increase in CART-IR in the mouse brain 2 h after administration.

2. Materials and methods

2.1. Animals

Male Imprinting Control Region (ICR) mice (28–30 g, Orient, Seoul, Korea) were handled daily for one week before the start of experiments. Animals were housed five per cage under standard conditions at 21–22 °C, with a 12 h light/dark cycle (lights on at 6:00 a.m.) and food and water provided ad libitum. All procedures were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and were approved by the Animal Care and Use Committee of Korea University.

2.2. Caffeine administration

Caffeine (Sigma–Aldrich Co., St. Louis, MO, USA) was dissolved in 0.9% saline. To assess time-dependent changes in CART-IR, we injected caffeine [100 mg/kg, intraperitoneally (i.p.)] to mice in a volume of 0.1 ml/10 g and sacrificed the mice at 0.25 h, 0.5 h, 1 h, 2 h, or 8 h post injection (n = 7 per group at each time point). Naïve untreated mice were used as a control (CON, n = 13/group) (Fig. 1). In addition, to assess dose-dependent changes in CART-IR, we injected mice with one of three doses of caffeine (25, 50, or 100 mg/kg, i.p., n = 7 per group) or saline (0.1 ml/10 g, i.p., n = 12) and sacrificed the mice 2 h later, at which time CART-IR was observed to reach its peak in the brain areas evaluated. Naïve untreated mice and mice injected with saline were used as a control and vehicle control, respectively (Fig. 1).

2.3. Tissue preparation and CART immunohistochemistry

All mice were anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and sacrificed via intracardial perfusion with 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.2. The brains were then removed, fixed in paraformaldehyde for 18 h, and placed in 20% glycerol/0.1 M sodium phosphate buffer overnight at 4 °C. A freezing microtome (Microm International GmbH, Walldorf, Germany) was used to prepare serial coronal sections (30 μm) of the whole brain. These sections were stored in a cryoprotectant [30% RNase-free sucrose, 30% ethylene glycol, and 1% polyvinylpyrrolidone (PVP-40) in 100 mM PPB, pH 7.4] at –20 °C until required.

Brain sections were selected to contain the cerebral cortex (1.54~1.42 mm from bregma), nucleus accumbens shell; AcbSh (1.10~0.98 mm from bregma), dorsal bed nucleus of the stria terminalis; dBNST (0.26~0.14 mm from bregma), central nucleus of the amygdala; CeA (–1.22~–1.34 from bregma), paraventricular hypothalamic nucleus; PVN (–0.70~–0.82 mm from bregma), arcuate hypothalamic nucleus; Arc (–1.46~–1.58 mm from bregma), anterior hypothalamic area; LHA (–1.34~–1.58 mm from bregma), zona incerta; ZI (–1.22~–1.34 mm from bregma), and locus coeruleus; LC (–5.34~–5.40 mm from bregma) according to the Paxinos and Franklin atlas (2003). Selected brain sections were incubated in 1% H_2O_2/phosphate buffered saline (PBS) for 10 min to block endogenous peroxidase activity. After washing with PBS, brain sections were incubated in 0.3% Triton X-100/PBS and 5% normal goat serum (Vector Laboratories, Burlingame, CA, USA) at room temperature for 30 min and then reacted for two days at 4 °C with anti-CART antibody (1:4000; Phoenix Pharmaceuticals, Burlingame, CA, USA). The specificity of CART antibody has been previously described (Dun et al., 2000; Jones and Kuhar, 2008; Kuhar and Yoho, 1999; Kuriyama et al., 2004). Brain sections were then incubated in biotinylated goat anti-rabbit IgG (1:500, Vector Laboratories) for 2 h at room temperature. After several washing steps with PBS, the brain sections were incubated in a solution containing an avidin–biotin–peroxidase complex (1:250, Vector Laboratories) for 1 h at room temperature. After rinsing, brain sections were reacted in a diaminobenzidine solution containing 0.3% H_2O_2 for 5 min, followed by several rinses with 0.01 M PBS. Finally, the slides were dried overnight and cover-slipped with Permount (Fisher Scientific, Pittsburgh, PA, USA).

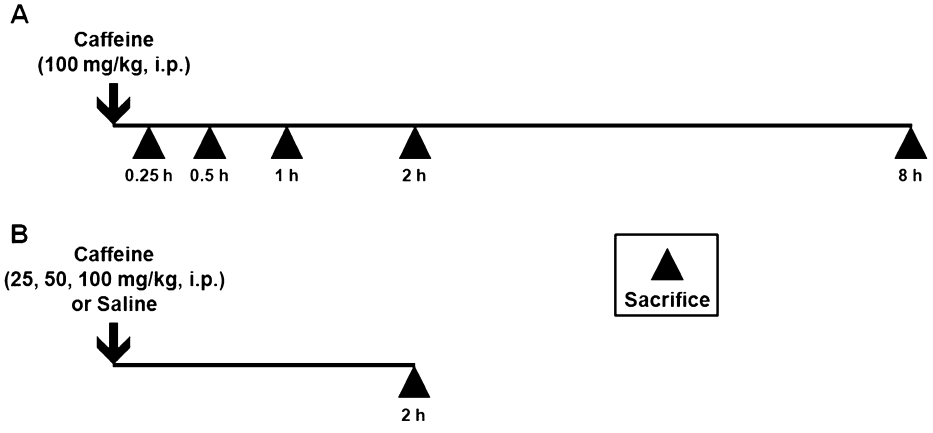

Fig. 1. Schematic representation of the experimental procedures and groups. A. Time-dependent changes in cocaine- and amphetamine-regulated transcript immunoreactivity (CART-IR) after caffeine administration. To assess time-dependent changes in CART-IR, mice were sacrificed at 0.25 h, 0.5 h, 1 h, 2 h, or 8 h after caffeine administration (100 mg/kg, i.p., n = 7 per group at each time point). Naïve untreated mice were used as a control (n = 13). B. Dose-dependent changes in CART-IR 2 h after caffeine administration. To assess dose-dependent changes in CART-IR, mice were injected with either one of three doses of caffeine (25, 50, or 100 mg/kg, i.p., n = 7 per group) or saline (0.1 ml/10 g, i.p., n = 12) and sacrificed 2 h later. Naïve untreated mice and mice injected with saline were used as a control and vehicle control, respectively.