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CART PEPTIDE IN THE NUCLEUS ACCUMBENS REGULATES PSYCHOSTIMULANTS: CORRELATIONS BETWEEN PSYCHOSTIMULANT AND CART PEPTIDE EFFECTS

MARTIN O. JOB [a,b]* **AND MICHAEL J. KUHAR** [a]

[a] *The Yerkes National Primate Research Center of Emory University, 954 Gatewood Rd NE, Atlanta, GA 30329, USA*

[b] *Psychobiology Section, Molecular Neuropsychiatry Research Branch, National Institute on Drug Abuse, Intramural Research Program, NIH, Baltimore, MD 21224, USA*

Abstract—In this study, we reexamined the effect of Cocaine-and-Amphetamine-Regulated-Transcript (CART) peptide on psychostimulant (PS)-induced locomotor activity (LMA) in individual rats. The Methods utilized were as previously published. The PS-induced LMA was defined as the distance traveled after PS administration (intraperitoneal), and the CART peptide effect was defined as the change in the PS-induced activity after bilateral intra-NAc administration of CART peptide. The experiments included both male and female Sprague-Dawley rats, and varying the CART peptide dose and the PS dose. While the average effect of CART peptide was to inhibit PS-induced LMA, the effect of CART peptide on individual PS-treated animals was not always inhibitory and sometimes even produced an increase or no change in PS-induced LMA. Upon further analysis, we observed a linear correlation, reported for the first time, between the magnitude of PS-induced LMA and the CART peptide effect. Because CART peptide inhibits PS-induced LMA when it is large, and increases PS-induced LMA when it is small, the peptide can be considered a homeostatic regulator of dopamine-induced LMA, which supports our earlier homeostatic hypothesis. Published by Elsevier Ltd on behalf of IBRO.

Key words: CART peptide, nucleus accumbens, psychostimulant, dopamine, locomotor activity, homeostasis.

INTRODUCTION

Cocaine-and-Amphetamine-Regulated-Transcript peptide (CART 55–102, CART peptide) is an active substance/neurotransmitter with a variety of effects throughout the body (Rogge et al., 2008; Zhang et al., 2012; Subhedar et al., 2014; Kuhar, 2016). In the brain, CART mRNA and peptides are found in many discrete nuclei including the nucleus accumbens (NAc), a region with a strong dopamine (DA) input (Douglass et al., 1995; Koylu et al., 1998), and exposure to cocaine (COC) or amphetamine (AMPH) increases the levels of CART mRNA in the NAc (Douglass et al., 1995; Albertson et al., 2004). Electron microscopic immunohistochemical data and confocal microscopic immunofluorescence data show that tyrosine hydroxylase-positive nerve terminals synapse on CART peptide-containing neurons in the NAc (Smith et al., 1999; Upadhya et al., 2012). Injection of COC increases the number of CART peptide-positive cells that co-stain for c-Fos in the NAc (Hubert and Kuhar, 2008).

There are additional data that support a CART peptide–DA interaction. For example, several studies have shown that CART peptide inhibits psychostimulant (PS)- and DA-induced behavioral effects, notably locomotor activity (LMA), and COC self-administration (Jaworski et al., 2003, 2008; Kim et al., 2003; Job and Kuhar, 2012; Job et al., 2012, 2013, 2014; Job, 2016). The bulk of these previous studies on CART peptide–DA interactions have involved an intra-NAc injection of CART peptide followed by other drug treatments and behavioral analyses. The mechanism of this inhibitory effect by CART peptide appears to require extracellular DA release (Job, 2016) and simultaneous activation of at least D1 and D2 dopamine receptors (Moffett et al., 2011). Also, PS-induced effects were altered in CART knockout mice (Couceyro et al., 2005), though not all studies agree (Moffett et al., 2006). More recent findings have been reviewed (Kuhar, 2016).

Intra-NAc CART peptide effect on DA-mediated activity is not always inhibitory. For example, the inhibition was lost after repeated doses of COC (Job et al., 2013). Also, intra-NAc CART peptide had no effect on LMA evoked by selective activation of DA D2 receptors in the NAc (Moffett et al., 2011). Interestingly, CART peptide may exert excitatory/facilitatory effects on DA-mediated activity. For example, in one study (Upadhya et al., 2012), administration of CART peptide antibody into the NAc shell suppressed food self-administration induced by systemic injection of a DA D2/D3 agonist implying that blockade of CART peptide action may result in inhibition of specific DA-mediated actions. In another study, intra-NAc CART peptide potentiated LMA evoked by intra-NAc injections of a selective DA D1 agonist (Moffett et al., 2011). These studies suggest that, under certain conditions, intra-NAc CART peptide may have no effect or enhance/facilitate some DA-induced behavioral effects.

*Correspondence to: M. O. Job, Psychobiology Section, Molecular Neuropsychiatry Research Branch, National Institute on Drug Abuse, Intramural Research Program, NIH, Baltimore, MD 21224, USA. Fax: +1-443-740-2829.
E-mail address: femijob@gmail.com (M. O. Job).

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The mechanism of CART peptide effect on PS is not very clear but it has been suggested that it is a homeostatic regulator of DA-mediated activity (Rogge et al., 2008). This implies that the effect of CART peptide should be related to the DA-mediated activity: when DA activity is high, CART peptide effect should be more inhibitory; when DA activity is low, CART peptide should be less inhibitory or even excitatory. In line with this idea, a previous report showed that COC-induced LMA was related to endogenous CART peptide levels in the NAc when individual subjects were considered (Job et al., 2012). In characterizing CART peptide function, it is important to determine, in individual subjects, whether this relationship occurs after exogenous administration of CART peptide into the NAc.

This study was undertaken to determine if the CART peptide effect is related to the PS effect. In this study, we again examine the effects of bilateral intra-NAc injections of CART peptide on COC- and AMPH-induced LMA. A major difference between this and earlier studies is that earlier studies looked at average changes in PS-induced activity after intra-NAc CART peptide administration, but the approach here was to consider the response of each individual animal. Thus, the relationship of CART peptide to the action of psychostimulants was reexamined.

EXPERIMENTAL PROCEDURES

Animals

Animal care was provided in accordance with the Emory University Institute of Animal Care and Use Committee and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. For this study, we used a total of fifty-eight Sprague-Dawley rats: thirty-two male and twenty-six female rats (Charles River Inc, Wilmington, MA). The rats had access to chow and water *ad libitum*, and were maintained on a 12-h light: dark cycle (lights on at 7 am). The males and females used in this study were age-matched: the male and female rats were aged 3.4–7.8 months and 4–6 months, respectively. The male and female rats weighed 400–700 g and 250–400 g, respectively, at the time of the experiments.

Stereotaxic surgery

The rats were surgically implanted with bilateral stainless steel guide cannulae (22 gauge; Plastics One, Roanoke, VA) to target the NAc, under isoflurane anesthesia, as previously described (Job et al., 2013, 2014). The implantation was done with the aid of a stereotaxic instrument (David Kopf, Tujunga, CA). The coordinate targets used were according to the rat brain atlas by Paxinos and Watson (1988) and were as follows: anterior-posterior (A/P) 1.6 mm, medial–lateral (M/L) ±1.5 mm and dorsal–ventral (D/V) −5.7 mm to place the tip of the guide cannulae at a point 2 mm above the NAc. The placement of the guide cannulae was done in this way to minimize damage to the NAc. After implantation, the bilateral guide assembly was secured to the skull via acrylic dental cement and 2–4 stainless steel screws. Bilateral dummy cannulae (obturators) that did not extend beyond the guide cannulae were inserted into the bilateral guide cannulae to prevent occlusion. After surgery, the rats were allowed to recover for at least 1 week before experimentation.

Drug administration

CART peptide (CART 55–102, American peptide Co, Sunnyvale CA) (or saline as a control) was bilaterally administered directly into the NAc. Both hemispheres were injected simultaneously. The dose of CART peptide injected into each hemisphere was 1.0 or 2.5 μg, depending on the experiment. Intra-accumbal infusions were done through stainless steel bilateral injector cannulae (28 gauge, Plastics One) inserted into the previously implanted bilateral guide cannulae. The injector cannulae were connected to two 25 μL microsyringes (Hamilton Co, Reno, NV) via polyethylene-50 (PE-50) tubing. The two microsyringes were driven by micropumps connected to a Micro4 Microsyringe Pump Controller (World Precision Instruments, Sarasota, FL). To inject, the rats were restrained gently, the obturator was removed and the injector cannulae were inserted in its place. When placed into the guide, the injector cannulae extended 2 mm beyond the end of the guide to access the NAc. Drug was bilaterally injected for 30 s, with an additional 30 s to allow the injected fluid to diffuse before removal of the injector cannulae. After removal of the injector cannulae, the obturator was placed back into the guide cannulae. Afterward, animals were immediately given systemic administration of PS, either COC (cocaine hydrochloride, NIDA) or AMPH (D-Amphetamine hemisulfate, Sigma–Aldrich, St Louis, MO) or saline (control). The systemic administration of PS (or saline) was done via the intraperitoneal (i.p.) route. In accordance with previous studies (Jaworski et al., 2003, 2008; Kim et al., 2003; Job and Kuhar, 2012; Job et al., 2012, 2013, 2014; Job, 2016), all bilateral intra-NAc and systemic drug and vehicle control injections were given in volumes of 0.5 μL/side and 1 mL/kg, respectively.

LMA testing

LMA testing was done in locomotor chambers. The locomotor chambers (Omnitech Electronics, Columbus, OH, USA) were 40 × 40 × 30 cm in dimension and made of transparent plexiglass walls and contained 32 photobeams located 5 cm above the floor. The locomotor chambers were connected to a computer equipped with software (Digipro, Omnitech Electronics) to measure LMA. On an experimental day, rats were placed into the locomotor chambers for 30 min to habituate to their surroundings before the recording of basal LMA. After 30 min of basal LMA recording, rats were removed from the chambers and pretreated with bilateral intra-NAc saline or CART peptide and systemic PS (or saline) and returned to the chamber. PS (or saline) was administered immediately after bilateral intra-NAc administration of CART peptide or saline. The