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Research Report

Comparative distribution of cocaine- and amphetamine-regulated transcript (CART) in the hypothalamus of the capuchin monkey (*Cebus apella*) and the common marmoset (*Callithrix jacchus*)

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ABSTRACT

Cocaine- and amphetamine-regulated transcript (CART) is widely distributed in the brain of many species. In the hypothalamus, CART neurotransmission has been implicated in diverse functions including energy balance, stress response, and temperature and endocrine regulation. Although some studies have been performed in primates, very little is known about the distribution of CART neurons in New World monkeys. New World monkeys are good models for systems neuroscience, as some species have evolved several behavioral and anatomical characteristics shared with humans, including diurnal and social habits, intense maternal care, complex manipulative abilities and well-developed frontal cortices. In the present study, we assessed the distribution of CART mRNA and peptide in the hypothalamus of the capuchin monkey (*Cebus apella*) and the common marmoset (*Callithrix jacchus*). We found that the distribution of hypothalamic CART neurons in these monkeys is similar to what has been described for rodents and humans, but some relevant differences were noticed. Only in capuchin monkeys CART neurons were observed in the suprachiasmatic and the intercalatus nuclei, whereas only in marmoset CART neurons were observed in

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Abbreviations: 3v, third ventricle; ac, anterior commissure; Arc, arcuate nucleus; AVPV, anteroventral periventricular nucleus; DAH, dorsal anterior nucleus; DMH, dorsomedial nucleus of the hypothalamus; fx, fornix; IC, intercalatus nucleus; LHA, lateral hypothalamic area; LM, lateral mammillary nucleus; ME, median eminence; MM, medial mammillary nucleus; MPA, medial preoptic area; mt, mammillothalamic tract; ot, optic tract; ox, optic chiasm; PeN, periventricular nucleus; PeP, posterior periventricular nucleus; PH, posterior nucleus of the hypothalamus; PMV, ventral premammillary nucleus; PPA, preoptic periventricular area; PVH, paraventricular nucleus of the hypothalamus; RCh, retrochiasmatic nucleus; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; SUM, supramammillary nucleus; VMH, ventromedial nucleus of the hypothalamus

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the dorsal anterior nucleus. We also found that the only in marmoset displayed CART neurons in the periventricular preoptic nucleus and in an area seemingly comprising the premammillary nucleus. These hypothalamic sites are both well defined in rodents but poorly defined in humans. Our findings indicate that CART expression in hypothalamic neurons is conserved across species but the identified differences suggest that CART is also involved in the control of species-specific related functions.

1. Introduction

Cocaine- and amphetamine-regulated transcript (CART) was identified by Douglass and coworkers as an mRNA whose expression in the rat striatum is enhanced by psychomotor stimulant drugs (Douglass et al., 1995). The CART peptides exist in two forms, a short form with 116 amino acids and a long form with 129 amino acids (Douglass et al., 1995). The rat CART cDNA sequence is 80% identical to the human cDNA with 92% homology observed in the protein-coding region. At the amino acid level, there is a 95% identity between rat and human; however, the human mRNA only exists in the short form (Douglass and Daoud, 1996). The receptor(s) for CART peptides have not been identified, but in vitro studies have shown that CART fragments bind to AtT20 cells and activate extracellular regulated kinase (ERK) signaling via a G-protein-coupled receptor (Lakatos et al., 2005; Vicentic et al., 2005, 2006).

Neuroanatomical studies in rodents have shown that CART and CART peptides are widely distributed throughout the brain (Couceyro et al., 1997; Koylu et al., 1997, 1998) and are one of the most abundant transcripts in the rodent hypothalamus (Gautvik et al., 1996). In the hypothalamus, CART is expressed mainly by neurons located in nuclei involved in energy balance and autonomic regulation; these nuclei include the arcuate nucleus (Arc), the dorsomedial nucleus (DMH), the paraventricular nucleus (PVH), and the lateral hypothalamic area (LHA) (Koylu et al., 1997). In these sites, CART neurons have been shown to coexpress different neuropeptides and neurotransmitters, such as proopiomelanocortin in the Arc, GABA and melanin-concentrating hormone in the LHA, and thyrotropin-releasing hormone and oxytocin in the PVH (Broberger, 1999; Elias et al., 2001; Vrang et al., 1999a). Hypothalamic CART neurons respond to and are regulated by circulating leptin—a hormone secreted by white adipose tissue that carries information to the brain regarding energy stores (Duan et al., 2007; Elias et al., 2000, 2001; Flier, 1998; Kristensen et al., 1998; Zhang et al., 1994). Lack of leptin (as in ob/ob mice) or its functional receptor (as in db/db mice) causes morbid obesity, hypogonadism and infertility (Ahima et al., 2000; Casanueva and Dieguez, 1999; Louis and Myers, 2007). Thus, CART is thought to be a relevant downstream target of leptin with a role in leptin's effects on energy balance and neuroendocrine regulation.

The broad distribution of CART in the brain suggests that it is also involved in several other functions. In fact, studies have established a link between CART neurotransmission and a vast array of physiological processes including reward and reinforcement (Adams et al., 1999; Dandekar et al., 2008a; Kuhar and Dall Vechia, 1999), feeding and satiety (Hunter et al., 2004; Kuhar et al., 2000, 2002; Rogge et al., 2008), depression and anxiety (Dandekar et al., 2008b; Wiehager et al., 2009), sleep (Mendez-Diaz et al., 2009), hypothermia (Skibicka et al., 2009), sensory processing (Cavalcante et al., 2006) and stress response (Dominguez et al., 2004; Koylu et al., 2006).

High levels of CART peptide and mRNA have also been detected throughout the human brain (Bai et al., 2005; Charnay et al., 1999; Douglass and Daoud, 1996; Elias et al., 2001; Hurd and Fagergren, 2000). However, because the availability of human brain tissue is low and its preservation difficult, neuroanatomical and physiological studies in nonhuman primates are of great value to the understanding of the structural and functional organization of the primate brain. Following this line, some species, including the capuchin monkey and the common marmoset, have evolved several behavioral and anatomical characteristics in common with humans. They display diurnal and social habits, their infants are born very immature, they show intense maternal care, they have complex manipulative abilities, and they have well-developed frontal lobes (Burman and Rosa, 2009; Dum and Strick, 2005; Fragaszy and Adams-Curtis, 1991, 1998; Fragaszy et al., 1991; Menezes et al., 1993; Ross et al., 2010; Souza de Oliveira et al., 1999). These commonalities make these New World monkeys good models for a comparative study of the structural organization of the primate brain. Thus, in the present study we describe and compare the distribution of CART neurons in the hypothalamus of two species of New World monkeys: the capuchin monkey (*Cebus apella*) and the common marmoset (*Callithrix jacchus*). We show here that, although high homology of CART across species (e.g., rats and humans) has been described, proximate species of primates (e.g., capuchin monkey and common marmoset) display unique patterns of hypothalamic CART distribution. CART expression is highly conserved across species in defined nuclei, but some identified differences suggested that CART may also be involved in the control of species-specific related functions.

2. Results

To determine the distribution of CART immunoreactivity in the hypothalami of the capuchin monkey and the common marmoset, we initially tested two different antisera: one made against the rat peptide (anti-rCART) and the other made against the human peptide (anti-hCART). We found that both antisera produced similar labeling patterns. Thus, for consistency, we chose to use the anti-rCART to perform our study.

To evaluate the specificity of the anti-rCART serum in monkey brain sections, the antiserum was preincubated in