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BRAIN RESEARCH

Research Report

Regulation of CART mRNA by stress and corticosteroids in the hippocampus and amygdala

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ARTICLE INFO

Article history:
Accepted 14 March 2007
Available online 20 March 2007

Keywords:
HPA axis
Anxiety
Depression

ABSTRACT

CART (Cocaine-Amphetamine-Regulated Transcript) has been shown to be regulated by corticosteroids in the hypothalamus, but its regulation by corticosteroids and stress has not been well examined in the hippocampus or the amygdala. Further, CART has been implicated in the transition to puberty. In this study we examine the effects of acute (30 min) stress on CART mRNA in prepubescent and adult rats. In addition, we examined chronic (21 day × 6 h) restraint stress upon the expression of CART mRNA in the hippocampus and the amygdala and the effects of 7 days of adrenalectomy and corticosteroid replacement upon CART expression in these regions of the adult rat brain. We found an up-regulation of CART mRNA in the central amygdala induced by acute but not chronic stress and an up-regulation in the dentate gyrus induced by chronic but not acute stress. Adrenalectomy reduced CART expression in the dentate gyrus but not the amygdala and this effect was blocked by corticosterone but not RU28,362 or aldosterone replacement, suggesting a synergism of mineralocorticoid and glucocorticoid receptors. Our data establish that CART expression is regulated by stress in a regionally and time specific manner and that CART is regulated by corticosteroid actions in the hippocampus.

1. Introduction

Stress and corticosteroids play a role in a number of neuropsychiatric disorders, particularly major depressive disorder. Both stress and corticosteroids have been linked to the structural changes seen in these disorders and in animal models such as chronic restraint stress (McEwen and Olie, 2005). CART (Cocaine-Amphetamine-Regulated Transcript) plays a role in a number of different behaviors, such as feeding and drug use (Hunter and Kuhar, 2003), and a number of studies have suggested that CART may have a role in the central response to stressful stimuli. Early anatomical work demonstrated its localization in a number of regions associated with stress and anxiety (Douglass et al., 1995; Couceyro et al., 1997; Koylu et al., 1997, 1998) which led to several studies demonstrating the anxiogenic effects of centrally injected CART peptides in the elevated plus maze and social interaction (Kask et al., 2000; Asakawa et al., 2001; Chaki et al., 2003; Stanek, 2006). More recently, it has been shown that human adolescents carrying the Leu34Phe substitution in the pro-CART peptide, had a higher level of anxiety and depression than control subjects (Miraglia Del Giudice et al., 2006). It seems likely that CART may have a role in anxiety and depression that is only beginning to be understood.

In parallel to the studies of CART's influence on anxious behaviors, a more substantial literature has demonstrated a number of interactions between CART and the HPA axis, especially in regard to corticosteroids. Central CART injection

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0006-8993/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2007.03.042

produces increased plasma corticosterone and ACTH (Vrang et al., 2000; Stanley et al., 2001) and, reciprocally, CART expression in the brain and blood is subject to regulation by corticosteroids (Balkan et al., 2001; Vrang et al., 2003; Vicentic et al., 2004; Hunter et al., 2005; Koylu et al., 2006). More recently Balkan has shown that a forced swim stress increases CART peptide levels in the amygdala of male rats while lowering it in females (Balkan et al., 2006). Other evidence implicates CART in the HPG axis as well as the HPA, particularly in the regulation of GnRH secretion (Lebrethon et al., 2000a,b; Parent et al., 2000) and, controversially, the onset of puberty (Adam et al., 2000; Lebrethon et al., 2000b; Brann et al., 2002). On this basis we decided to examine changes in the levels of CART mRNA in the hippocampus and amygdala following acute stress in adolescent and adult animals and the effects of chronic stress and corticosteroid manipulations in adults.

2. Results

2.1. Acute stress

Based upon the aforementioned findings that CART may have a role in the onset of puberty in rodents we chose to analyze the effect of acute stress upon both adult and prepubertal animals. Analysis of CART mRNA expression in the dentate gyrus showed a significant effect of age ($n=6$, F (1,18) = 10.66) but not stress with higher levels in unstressed prepubertal animals than adults in either condition (see Fig. 1) and a trend towards higher levels in the stressed condition.

In the central amygdala, a similar pattern emerged with regard to age, though not to stress. There was a significant main effect of age (F (1,16) = 59.93) such that both stressed and unstressed prepubertal animals showed substantially higher CART mRNA expression than unstressed adults. There was also a significant main effect of stress (F (1,16) = 9.20) which was observed in adult animals but not the prepubertal rats. This increase did not produce CART mRNA levels comparable to those of prepubertal animals (see Fig. 2), suggesting the possibility that the younger animals may be subject to a ceiling effect. Corticosterone levels for these animals was reported in Romeo et al. (2004a) where it was shown that peak levels of corticosterone did not differ between adults and prepubescent rats, though the younger animals showed a longer elevation in corticosterone levels than the adults.

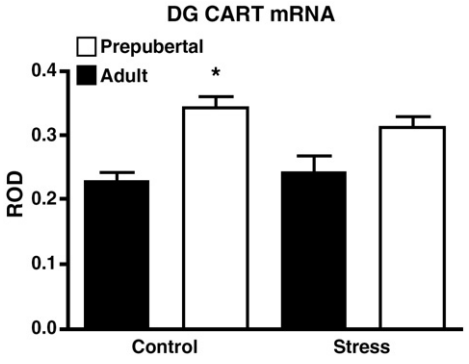

Fig. 1 – Relative optical density (ROD) of CART mRNA expression (±S.E.M.) in the dentate gyrus of adult and prepubertal rats at baseline and 2 h after a 30 min restraint stress. Prepubertal rats showed significantly (F (1,18) = 10.66) higher levels of CART mRNA expression than adults, though no effect of stress was observed at either age. Asterisk indicates a significant difference between unstressed adult and prepubertal rats ($n=6$, $p<0.05$).

2.2. Chronic stress

We followed up our acute stress studies with an examination of the effects of chronic restraint stress on adult male rats. Prepubertal animals were not included due to the absence of an effect in the acute stress study and due to the fact that the chronic restraint stress paradigm would include most if not all of the period of puberty.

In contrast to the acute stress study, we found that chronic restraint stress increased the expression of CART mRNA in the dentate gyrus by more than 85% (±20%, $p<0.05$, see Fig. 3) and had no impact on CART mRNA levels in the central amygdala (Fig. 4).

2.3. Adrenalectomy and corticosteroid treatment

There was a significant main effect of treatment (F (4,30) = 5.62) in the dentate (Fig. 5) but no effect in the central amygdala (Fig. 6). Adrenalectomy reduced CART message by more than 50% (±12%, $p<0.05$). Neither the selective MR agonist aldosterone nor the selective GR agonist RU28,362, given alone, reversed this effect, but treatment with corticosterone, which occupies both MR and GR, did restore CART mRNA expression in the dentate.

3. Discussion

Our results demonstrate that stress alters CART mRNA expression in a regionally specific manner in response to stress duration. In addition we show that acute stress has no impact on CART expression in adolescent animals relative to adults, though this may be a ceiling effect. We also show that CART is regulated by chronic corticosterone and adrenalectomy in the hippocampus but not in the amygdala by mechanisms potentially involving both MR and GR.

We first examined the effect of an acute restraint stress on CART mRNA expression in the prepubertal and adult rat brain. We found that acute stress elevates CART mRNA expression in the central amygdala of adults but not prepubertal animals. The change observed in the adults is in agreement with Balkan's observations of CART peptide levels after an acute swim stress (Balkan et al., 2006). The elevated levels of CART mRNA in the prepubertal animals, which did not respond to stress, suggest that CART may contribute to the differences in stress response between this age group and adults (Romeo et al., 2004a,b, 2006), though only further study will establish the actual relationship between CART and the juvenile stress response.

Our confirmation of Balkan's result led us to examine whether chronic stress had the same impact on CART expression. The effect of chronic stress was in fact quite