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Research Report

Forced swim stress elicits region-specific changes in CART expression in the stress axis and stress regulatory brain areas

Burcu Balkan[a,b,*], Aysegul Keser[a,b], Oguz Gozen[a,b], Ersin O. Koylu[a,b], Taner Dagci[a,b], Michael J. Kuhar[b,c], Sakire Pogun[b]

[a]Ege University School of Medicine Department of Physiology, Izmir, Turkey
[b]Ege University Center for Brain Research, Izmir, Turkey
[c]Yerkes National Primate Research Center of Emory University, Atlanta, GA, USA

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ABSTRACT

CART mRNA and peptides are highly expressed in the anatomical structures composing the hypothalamo-pituitary-adrenal (HPA) axis and sympatho-adrenal system. Anatomical and functional studies suggest that CART peptides may have a role in the regulation of the neuroendocrine and autonomic responses during stress. Our previous study showed that CART peptides increased significantly in the male hypothalamus and amygdala 10 min after the forced swim stress. The present study aimed to examine the effect of forced swim stress on CART peptide expression in selected brain regions, including those where CART peptide expression has not been reported before (frontal cortex, pons, medulla oblongata), as well as in endocrine glands related to stress in male Sprague Dawley rats. A total of 16 (n=8) animals were used, including control groups. Rats were subjected to forced swim on two consecutive days, and sacrificed on the second day, 2 h after the termination of the stress procedure. Frontal cortex, pons, medulla oblongata, hypothalamus, pituitary and adrenal glands were dissected and homogenized. CART peptide expression in these tissues was measured by Western Blotting and six different CART peptide fragments were identified. Our results showed that forced swim stress elicited region-specific changes in CART peptide expression. CART was upregulated in the frontal cortex, hypothalamus, medulla oblongata and adrenal gland while there was no change in the pons and pituitary. Enhanced CART peptide fragments in these brain regions and adrenal glands may have a role in the regulation of the HPA and sympatho-adrenal axis activity during stress response.

1. Introduction

Cocaine and amphetamine regulated transcript (CART) mRNA and peptides are abundant and widely distributed in the brain and endocrine glands (Couceyro et al., 1997; Douglass et al., 1995; Jensen et al., 1999; Kobayashi et al., 2004; Koylu et al., 1997, 1998). Peptides derived from CART mRNA have been implicated in a number of physiological processes such as reward and reinforcement (Hunter and Kuhar, 2003; Rogge et al., 2008), feeding (Kristensen et al., 1998), endocrine and autonomic regulation (Dun et al., 2006; Fekete and Lechan, 2006; Koylu et al., 2006; Wierup and Sundler, 2006) and anxiety (Stanek, 2006).

The hypothalamo-pituitary-adrenal (HPA) axis is one of the key components activated during the stress response.

* *Corresponding author at:* Ege University, School of Medicine, Department of Physiology, Izmir, 35100, Turkey. Fax: +90 232 3746597.
E-mail address: burcu.balkan@ege.edu.tr (B. Balkan).

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CART mRNA and peptides are highly expressed in the HPA axis and in the brain regions [limbic regions: prefrontal cortex, hippocampus, amygdala; brain stem regions: the nucleus of the solitary tract (NTS) and parabrachial nucleus] which regulate the HPA axis activity (Couceyro et al., 1997; Douglass et al., 1995; Koylu et al., 1997, 1998; Vrang et al., 1999b). In the hypothalamus, highest densities of CART stained cells are present in the paraventricular (PVN), supraoptic (SON) and arcuate nuclei (ARC). Especially, medial parvocellular PVN, which harbors neuroendocrine cells, expresses CART immunoreactive (IR) neurons. In the pituitary, anterior lobe exhibits moderate levels of CART-IR cells and the posterior lobe contains high density of CART-IR axonal fibers (Koylu et al., 1997). Functional studies clearly demonstrate that CART regulates the HPA axis activity. Intracerebroventricular (ICV) CART injections induce c-Fos expression in corticotropin releasing hormone (CRH) expressing neurons of the PVN (Vrang et al., 2000). Furthermore CART peptides increase CRH release from hypothalamic explants (Stanley et al., 2001). In agreement with this finding, ICV and PVN CART injections elevate adrenocorticotropic hormone (ACTH) and corticosterone (CORT) levels in blood (Stanley et al., 2001). In addition, studies show that hypothalamic CART expression is regulated by CORT (Balkan et al., 2001, 2003; Vrang et al., 2003).

Sympatho-adrenal system is another key component which is activated during the integrated stress response. Presympathetic neurons which control the activity of sympatho-adrenal system reside in the PVN (dorsal parvocellular region), ARC, lateral hypothalamic and retrochiasmatic (RCA) areas of hypothalamus, parabrachial nucleus, locus ceruleus, A7, A5 and C1 areas, rostral ventrolateral medulla (RVLM), medullary raphe and the nucleus of the solitary tract (NTS) (Palkovits, 2010; Saper, 2002). These hypothalamic areas and brain stem regions contain varying densities of CART expressing neurons (Fekete et al., 2004; Koylu et al., 1997, 1998). Retrograde tracing studies showed that CART-IR neurons in the ARC, RCA and RVLM project to the intermediolateral cell column (IML) in the spinal cord (Dun et al., 2002; Elias et al., 1998). Furthermore, anatomical structures composing the sympatho-adrenal axis highly express CART mRNA and peptides. Sympathetic preganglionic neurons which reside in the IML of the spinal cord are CART-positive (Dun et al., 2000b; Koylu et al., 1998). CART-IR is present especially in cardiovascular preganglionic neurons (Gonsalvez et al., 2010). In the adrenal gland, only the medulla contains cells which express CART mRNA and peptides (Couceyro et al., 1997; Koylu et al., 1997). Additionally, CART-IR fibers form a plexus under the adrenal capsule and they terminate in the medulla (Dun et al., 2000a).

Numerous studies examining the involvement of CART in the regulation of the HPA axis and sympatho-adrenal system have shown that CART may have an important role in the stress response (reviewed in Koylu et al., 2006). Furthermore, various systemic stress procedures change CART expression in the brain regions which regulate the stress response (Balkan et al., 2006; Dandekar et al., 2009; Gozen et al., 2007; Hindmarch et al., 2008; Hunter et al., 2007; Kang et al., 2010; Kong et al., 2003; Larsen et al., 2003; Orsetti et al., 2008; Ruginsk et al., 2011; Sergeyev et al., 2001; Yoo et al., 2011). Forced swim test, developed by Porsolt et al. (1977) is used as a psychophysical stressor which activates the HPA axis and results in endocrine, immune and neurochemical changes that accompany the stress response (Connor et al., 1997; Dayas et al., 2001; Veenema et al., 2003). When compared to restraint stress, forced swim activates a larger proportion of the cells in the brain regions which regulate the sympatho-adrenal system (Dayas et al., 2001; Liu et al., 2007). Furthermore, when the same exposure period is used, forced swim activates the pituitary and elevates the CORT levels more than restraint (Hueston et al., 2011; Jortner, 2008). In a previous study, we have shown that forced swim increases CORT and ACTH levels significantly in Sprague Dawley rats (Gozen et al., 2007). Some studies employ two-day forced swim to trigger the stress response (Banerjee et al., 2010; Calvez et al., 2011; Schindler et al., 2010; Tejedor-Real et al., 2007). Additionally, there are studies which show that forced swim has long term (24 h after the forced swim) effects on stress related peptides and mRNAs (Gesing et al., 2001; Veenema et al., 2003). Furthermore two-day forced swim upregulates CART peptide expression in the stress-regulatory regions, hypothalamus and the amygdala and increases CART mRNA in the central nucleus of amygdala and in locus ceruleus (Balkan et al., 2006; Gozen et al., 2007; Kang et al., 2010). Therefore we selected 2-day forced swim protocol to observe both the long term (24 h after the first swim) and also the short term (2 h after the second swim) effects of the forced swim on CART expression.

The present study aimed to examine the effect of forced swim stress on CART peptide expression in the stress axis (hypothalamus, pituitary and adrenal gland) and stress-regulatory brain regions (frontal cortex, pons and medulla oblongata) of male rats. Different CART-IR bands specifically involved during the stress response were demonstrated by using quantified Western Blot analysis. Our approach was to find out if there is a change in total CART peptide expression, as well as to illustrate which CART-IR bands are specifically involved. We hypothesized that forced swim stress will increase different CART-IR bands in different brain regions and endocrine glands.

2. Results

In the present study, we labeled six different CART peptide fragments in all tissues examined: two bands in the range of 6.5–3.4 kD and four bands in the range of 14.3–6.5 kD (Fig. 1). CART-IR bands observed in the hypothalamus and pituitary were similar: two bands in the range of 6.5–3.4 kD and three bands in the range of 14.3–6.5 kD (Fig. 1). The two bands in the range of 6.5–3.4 kD were the most intense. Pons and medulla oblongata also expressed similar CART-IR bands: two intense bands in the range of 6.5–3.4 kD and two other bands in the range of 14.3–6.5 kD (Fig. 1). The highest molecular weight band observed in the hypothalamic and pituitary extracts was not detected in the brain stem extract. In the adrenal gland, we observed five CART-IR bands: one faint band in the range of 6.5–3.4 kD and four bands in the range of 14.3–6.5 kD (Fig. 1). The highest molecular weight band expressed in the adrenal gland was not observed in the other tissues. In the frontal cortex, there was one intense CART-IR band in the