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Region- and sex-specific changes in CART mRNA in rat hypothalamic nuclei induced by forced swim stress

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Abstract

Cocaine and amphetamine regulated transcript (CART) mRNA and peptides are highly expressed in the paraventricular (PVN), dorsomedial (DMH) and arcuate (ARC) nuclei of the hypothalamus. It has been suggested that these nuclei regulate the hypothalamic-pituitary-adrenal (HPA) axis, autonomic nervous system activity, and feeding behavior. Our previous studies showed that forced swim stress augmented CART peptide expression significantly in whole hypothalamus of male rats. In another study, forced swim stress increased the number of CART-immunoreactive cells in female PVN, whereas no effect was observed in male PVN or in the ARC nucleus of either sex. In the present study, we evaluated the effect of forced swim stress on CART mRNA expression in PVN, DMH and ARC nuclei in both male and female rats. Twelve male (stressed and controls, n=6 each) and 12 female (stressed and controls, n=6 each) Sprague-Dawley rats were used. Control animals were only handled, whereas forced swim stress procedure was applied to the stressed groups. Brains were dissected and brain sections containing PVN, DMH and ARC nuclei were prepared. CART mRNA levels were determined by in situ hybridization. In male rats, forced swim stress upregulated CART mRNA expression in DMH and downregulated it in the ARC. In female rats, forced swim stress increased CART mRNA expression in PVN and DMH, whereas a decrease was observed in the ARC nucleus. Our results show that forced swim stress elicits region and sex-specific changes in CART mRNA expression in rat hypothalamus that may help explain some of the effects of stress.

Keywords

CART; stress; hypothalamus

1. Introduction

The hypothalamus is a key brain region for the integration of endocrine and autonomic functions and energy homeostasis. Cocaine and amphetamine regulated transcript (CART)

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and CART-derived peptides are abundantly expressed in the rat and human hypothalamus (Dall Vechia et al. 2000; Douglass et al. 1995; Elias et al. 2001; Hurd and Fagergren 2000; Koylu et al. 1997; Kuhar and Yoho 1999). CART has been implicated in various physiological functions including the stress response, sympathetic activity and food intake (Dun et al. 2006; Koylu et al. 2006; Kuhar et al. 1999; Rogge et al. 2008).

In the hypothalamus, the paraventricular (PVN), dorsomedial (DMH) and arcuate (ARC) nuclei regulate the hypothalamic-pituitary-adrenal (HPA) axis (Herman et al. 2003), autonomic nervous system (Fontes et al. 2011; Palkovits, 1999) and feeding behavior (Elmquist et al. 1999; Myers et al. 2009). The PVN (parvocellular and magnocellular subdivisions), DMH and ARC contain high to moderate numbers of CART-immunoreactive neurons and axonal fibers (Koylu et al. 1997). In the parvocellular PVN, CART-immunoreactive neurons are located in the medial areas, which harbor neuroendocrine corticotropin releasing hormone (CRH)-synthesizing cells, as well as in the dorsal areas, which harbor presympathetic neurons projecting to the intermediolateral (IML) cell column of the spinal cord (Koylu et al. 1997; 1998). Parvocellular CRH neurons in the PVN receive regulatory input from γ-aminobutyric acid (GABA)-containing neurons in the DMH and proopiomelanocortin (POMC)-containing neurons in the ARC nucleus (Herman et al. 2003). CART is highly colocalized with GABA in the DMH neurons (Elias et al. 2001) and with POMC in the ARC neurons (Elias et al. 1998). CART-immunoreactive axon terminals, some of which originate in the POMC neurons of ARC (Fekete et al. 2004; Wittmann et al. 2005), end in close proximity to the soma and dendrites of CRH neurons (Sarkar et al. 2004; Vrang et al. 2000). CART synthesized or released in the PVN regulates HPA axis activity. It induces c-Fos expression (Vrang et al. 2000) and phosphorylation of cyclic adenosine 5'-monophosphate response element binding protein in the paraventricular CRH neurons, suggesting the regulation of CRH at the transcriptional level (Sarkar et al. 2004). CART peptides increase CRH release from the hypothalamus and elevate adrenocorticotropic hormone (ACTH) and corticosterone (CORT) levels in blood when injected into the cerebral ventricles (ICV) or PVN (Stanley et al., 2001). CART peptide injection also increases heart rate and blood pressure (Matsumura et al. 2001) and regulates feeding behavior (Abbott et al. 2001; Kristensen et al. 1998; Vrang et al. 1999) when administered ICV or into discrete hypothalamic nuclei.

Forced swimming is a psychophysical stressor (Calvez et al. 2011; Dayas et al., 2000), which results in neurochemical and endocrine changes that accompany the stress response (Dayas et al., 2000; Gozen et al. 2007; Hueston et al. 2011). Additionally, forced swim stress also triggers an increase in the sympathetic outflow to the cardiovascular system and elevates heart rate and blood pressure (Michalkiewicz et al. 2003). Studies (Diane et al. 2008; Liu et al. 2007) also show that forced swim changes the activity of feeding circuitry and inhibits food intake.

Stress regulates CART expression in different brain regions (Balkan et al., 2006, 2012; Dandekar et al., 2009; Gozen et al., 2007; Hunter et al., 2007; Kang et al., 2010; Kong et al., 2003; Orsetti et al., 2008; Ruginsk et al., 2011; Sergeyev et al., 2001). Few studies have investigated the regulation of hypothalamic CART during psychological stress.Dandekar et al. (2009) showed that social isolation reduced CART immunoreactivity levels in the PVN and ARC neurons. Also,Kang et al. (2010) reported that forced swim stress did not regulate CART mRNA expression levels in the PVN and ARC nucleus. These two studies were performed only in male rats. On the other hand, our previous studies demonstrated that hypothalamic CART peptides are regulated by forced swim stress and that this regulation is sex-specific. Western Blot analysis of the whole hypothalamus showed that forced swim stress increased CART peptide levels in male rats, whereas no effect was observed in females (Balkan et al. 2006, 2012). Furthermore, immunohistochemical analysis of the

hypothalamus showed that forced swim increased CART-immunoreactive cell numbers in the female PVN, whereas no effect was observed in the male PVN or in the ARC nucleus of either sex (Gozen et al. 2007).

Psychological stress may regulate CART expression at different levels ranging from transcription, to translation and secretion. In our previous reports, we demonstrated an increase in hypothalamic CART peptide expression reflecting a possible regulation at all levels. The present study aimed to examine the effects of forced swim stress on CART transcription in the hypothalamic nuclei of male and female rats. To the best of our knowledge, there is only one previous study (Kang et al 2010) that investigated the regulation of hypothalamic CART mRNA expression by psychological stress. This study examined only the male PVN and ARC nuclei and showed no effect. However, based on our previous reports, we hypothesized that forced swim stress will change CART mRNA expression in discrete hypothalamic nuclei, and that this effect will be sex-specific. In order to obtain the maximal effect of estradiol on hypothalamic CART expression, female rats in estrus were included in this study. Additionally, CART regulation by stress in the DMH has never been studied before. This is the first study that investigates the sex-specific regulation of CART mRNA in the PVN, DMH and ARC nuclei by stress.

2. Results

CART mRNA levels in the PVN, DMH and ARC nuclei were examined by in situ hybridization in four groups (n=6 in all groups) of rats using a 2×2 factorial design with sex (male, female) and stress (control, forced swim stress) as factors. Film autoradiograms of coronal sections through the PVN, DMH and ARC nuclei are shown in Figures 1, 2 and 3. In control groups, no significant sex difference was found in basal CART mRNA levels expressed in three hypothalamic nuclei. Following forced swim stress, we observed region-specific and sex-specific changes in CART mRNA expression in these nuclei (Figures 1, 2, 3 and 4). Forced swim stress increased CART mRNA significantly in female PVN ($p<0.05$), while there was no effect in male PVN (Figures 1 and 4). Additionally, there was a significant sex difference between the stressed groups in the PVN ($p<0.05$). On the other hand, forced swim stress increased CART mRNA significantly in the DMH of both sexes ($p<0.05$ in males; $p<0.01$ in females) (Figures 2 and 4). In contrast, CART mRNA levels were decreased significantly by stress in the ARC nucleus of both male and female rats ($p<0.0005$ in males; $p<0.05$ in females) (Figures 3 and 4).

3. Discussion

This study showed that forced swim stress elicits region- and sex-specific changes in CART mRNA expression in the rat hypothalamus. In male rats, forced swim stress upregulated CART mRNA expression in the DMH, whereas a downregulation was observed in the ARC. On the other hand, in female rats, CART mRNA expression was increased in the PVN and DMH, whereas it was decreased in the ARC nucleus following forced swim stress. In control groups, there was no significant sex difference in basal CART mRNA levels expressed in three hypothalamic nuclei studied. There is one study reporting no change in CART mRNA levels in the PVN and ARC nucleus of male rats following forced swim (Kang et al. 2010). In this study, only the optical density of the CART signal was taken into consideration during data analysis. However, in our study, CART signals were quantified as the area of expression × the optical density.

Our current results agree with our previous study (Gozen et al. 2007), which showed that forced swim stress increased CART-immunoreactive cell numbers significantly in the female PVN, whereas no change was observed in the male PVN. Based on the observations

obtained from these two studies, we can suggest that forced swim stress regulates PVN CART expression at transcription and/or translation levels in females, whereas there is no regulatory effect in males. In the ARC nucleus, following the same stress paradigm, no change in CART-immunoreactive cell numbers was reported in male or female rats (Gozen et al. 2007). However, the present study showed that forced swim stress downregulated CART mRNA in both sexes. This difference could reflect the time delay between CART transcription and CART translation; in other words, reduced CART transcription may have not yet affected CART translation.

Studies report that CART increases CRH release from hypothalamic explants (Stanley et al., 2001) and elevates blood ACTH and CORT levels when injected ICV (Vrang et al. 2000) or into the PVN (Stanley et al., 2001). CRH neurons may be activated by CART synthesized in the paraventricular CART neurons or released from CART-immunoreactive axonal fibers originating in other hypothalamic nuclei and brainstem areas (Fekete et al. 2004; Koylu et al. 1997; Wittmann et al. 2005). In the ARC, high levels of CART are expressed in the POMC neurons (Elias et al. 1998). In the DMH, CART is highly colocalized with melaninconcentrating hormone (MCH) (Broberger, 1999; Elias et al. 2001) and GABA (Elias et al. 2001). Studies show that CART/POMC neurons innervate the PVN (Fekete et al. 2004; Wittmann et al. 2005).Wittmann et al. (2005) reported that CART/α-melanocyte-stimulating hormone (peptide product of POMC gene) immunoreactive axons were mainly concentrated in the autonomic subcompartments (the dorsal, and the ventral parvocellular PVN), whereas the density of the fibers was less frequent in the neuroendocrine subcompartment (the medial parvocellular PVN). Similarly,Fekete et al. (2004) reported that some CART/MCH-immunoreactive neurons in the DMH directly innervated the PVN. All of these signaling molecules that are co-expressed with CART have been suggested to regulate the HPA axis (Herman et al. 2003; Kennedy et al. 2003). In our study, forced swim stress increased CART mRNA levels in the PVN of females and DMH of both sexes. The results of the current study suggest that forced swim stress upregulates CART synthesis in the female PVN and also increases paraventricular CART release from the axons originating in the DMH of both sexes. Increased paraventricular CART may activate CRH neurons enhancing the HPA axis activity.

Studies show that the PVN (Palkovits, 1999; Pyner and Coote 2000), DMH (Fontes et al. 2001, 2011; Thompson et al. 1996) and ARC (Ciriello et al. 2003; Elias et al. 1998; Li et al. 2009; Sim et al. 1991) neurons project to the key autonomic centers such as rostral ventrolateral medulla, periaquaductal gray, parabrachial nucleus, dorsal vagal complex, nucleus ambiguus and IML cell column of the spinal cord. All of these regions contain high/moderate densities of CART-IR axonal fibers (Dun et al. 2002; Elias et al. 1998; Koylu et al. 1998). Functional studies show that ICV (Matsumura et al. 2001) and intracisternal (Hwang et al. 2004) injections of CART peptide increase heart rate, arterial blood pressure, renal sympathetic nerve activity and plasma adrenaline levels, whereas intrathecal (Scruggs et al. 2005) CART peptide administration enhances the pressor response induced by glutamate. In addition, local CART peptide injections into the nucleus of the solitary tract attenuate phenylephrine-induced bradicardia (baroreflex) (Scruggs et al. 2003). Studies show that forced swim stress elevates heart rate and blood pressure (Michalkiewicz et al. 2003). During forced swim stress, increased CART synthesis in the PVN of females and the DMH of both sexes may increase CART release in the autonomic centers, which in turn, may enhance the sympathetic outflow to the cardiovascular system. Direct correlations between CART mRNA expression and autonomic nervous system activity during stress can be studied in the future. The PVN, DMH and ARC nuclei are also implicated in the regulation of feeding behavior and energy homeostasis (Abbott et al. 2003; Elmquist et al. 1999; Myers et al. 2009). Neural projections from the ARC CART/POMC neurons are distributed to other feeding-regulatory nuclei, including the PVN, DMH and the lateral hypothalamic area

(LHA) (Elias et al., 1999; Elmquist et al. 1999; Fekete et al. 2004; Simerly, 2004; Wittmann et al. 2005). Additionally, CART/POMC neurons in the ARC nucleus directly innervate the IML cell column of the spinal cord (Elias et al. 1998). These projections are suggested to regulate thermogenesis and energy expenditure. Interestingly, studies report contradicting effects of CART on food intake when administered ICV or into discrete hypothalamic nuclei. ICV CART injections reduce feeding (Kristensen et al. 1998; Vrang et al. 1999), while injections into the ARC, PVN, DMH or LHA increase feeding (Abbott et al. 2001; Kong et al. 2003). Also, CART overexpression in the ARC (Kong et al. 2003) and PVN (Smith et al. 2008) results in hyperphagia and increases body weight. Studies (Diane et al. 2008; Liu et al. 2007) show that acute stress inhibits feeding behavior. During forced swim stress, decreased CART mRNA levels in the ARC nucleus may reduce CART release in the PVN, DMH and LHA causing the anorectic effect that accompanies the acute stress response.

One of the major findings of this study is that although there was no sex difference between the basal CART mRNA levels expressed in the PVN, forced swim stress exerted sex-specific effects on paraventricular CART expression: CART mRNA was elevated in females in estrus, whereas no significant effect was observed in males. PVN contains estrogen receptors (Jennes and Langub 2000; Bingham et al. 2006). Although not demonstrated, paraventricular CART neurons may express estrogen receptors. Direct and indirect regulation of paraventricular CART mRNA by estradiol is possible. Functional studies suggest that estradiol (Dandekar et al. 2012; Silva et al. 2010) increases CART mRNA and peptide expression in the PVN. Towards the end of proestrus and during the onset of estrus, high blood estradiol levels are observed in female rats (Spornitz et al. 1999). Studies report that estradiol levels both in the plasma and in the PVN increase further during acute stress in proestrus and estrus (Liu et al. 2011). Additionally, some studies report that acute stress upregulates estrogen receptor expression in the PVN and brainstem nuclei (Estacio et al. 1996 and 2004; Liu et al. 2011). There is a pronounced sex difference in the regulation of the HPA axis. Estradiol increases HPA axis activity, while testosterone inhibits it (Lund et al. 2004; Williamson et al. 2005). Females have higher basal and stress-induced ACTH and CORT levels compared to males (Gozen et al. 2007; Solomon and Herman 2009). Sex differences in stress-related endocrine responses have been shown using different modulators; for example, nicotine increased the arginine-vasopressin response in males and the ACTH and CORT responses in females (Rhodes et al, 2001). Upregulation of paraventricular CART mRNA only in the stressed females may result in higher activation of CRH cells contributing to the sex difference observed in the stress-induced ACTH and CORT levels. We have used intact female rats, which were in the high-estrogen phase of the estrus cycle. Therefore, the mediation of estrogen in the observed sex difference is plausible. Future studies, aimed at elucidating the effects of gonadal hormones on CART mRNA expression (gonadectomies and hormone replacement), will lead to a better understanding of the underlying mechanism of the observed sex differences.

Anatomical and functional studies suggest that CART regulates the HPA axis, autonomic nervous system activity and feeding behavior. However, the data concerning the nature of this regulation is either inadequate or controversial. While CART receptors have been identified by binding and signaling studies, they have not yet been cloned (Lin et al. 2011; Rogge et al, 2008). The existence of multiple CART receptors linked to different intracellular signaling pathways is plausible. Based on this possibility, there may be distinct neural circuits within the hypothalamus in which CART acts oppositely (eg. excitatory/inhibitory). CART neurons in the PVN and DMH may form parts of the neural circuits that regulate the HPA axis and sympathetic activity. Therefore, increased CART synthesis in the DMH and the female PVN may increase the activity of the HPA axis and the autonomic nervous system during stress. On the other hand, CART neurons in the ARC may form a

part of the feeding circuit so that decreased CART synthesis in the ARC and subsequent decreased CART release in the PVN, LHA and DMH may reduce food intake during stress. This is the first study to demonstrate that CART transcription is regulated by psychological stress in the PVN, DMH and ARC nuclei of the hypothalamus, and that this regulation is sex-specific. The present findings will improve our understanding of the role of CART during the stress response and in stress-related diseases.

4. Experimental Procedure

4.1. Experimental Animals

Adult, male (250–300 g) and female (200–250 g) Sprague-Dawley rats were used in the study. Rats were housed (3–4 rats/cage) in standard plastic cages with food and water provided ad libitum during the habituation period for two weeks. Animals were maintained on 12:12 h light:-dark cycle (lights on 07:00–19:00). Manipulations of the rats were performed under the rules of the Institutional Animal Ethics Committee of Ege University, Izmir, Turkey, complying with the European Communities Council Directive (2003/003) and guided by the “International Guiding Principles for Biomedical Research Involving Animals” developed by the Council for International Organizations of Medical Sciences (NIH).

4.2. Experimental groups

Adult rats were assigned into four groups using a 2 × 2 factorial design with sex (male, female) and stress (control, forced swim stress) as factors. Control animals were only handled, whereas the forced swim stress procedure was applied to the stressed groups. In all female rats, vaginal smears were obtained on the first day (before forced swim in the stressed group) and on the second day (after sacrifice) to identify the phase of the estrus cycle. Only female rats in estrus (six controls, six stressed) were selected for the study. Twenty four rats (n=6, for each group) were used for the in situ hybridization procedure.

4.3. Forced swim procedure

In related literature, one- or two-day forced swim stress is used to trigger an acute stress response (Calvez et al. 2011; Dayas et al. 2000). We selected the 2-day forced swim protocol to observe both the long-term (24 hours after the first swim) as well as the short-term (10 minutes after the second swim) effects of forced swim on CART expression. Rats were forced to swim in water at 25°C, in a Plexiglas cylinder (diameter: 30 cm, height: 50 cm), for two consecutive days between 09:00 and 11:00 h; the duration of the swim was 15 min the first day and 6 min the second day (Porsolt et al., 1977). Water depth (20±2 cm) in the cylinder was adjusted to the size of the rat. We have previously shown that this stress procedure elevated blood ACTH and CORT levels significantly in male and female Sprague-Dawley rats (Gozen et al., 2007).

The two-day forced swim is used to trigger the stress response in many studies (Calvez et al., 2011; Schindler et al., 2010; Tejedor-Real et al., 2007; Banerjee et al., 2010). The two-day forced swim protocol used in this study has previously been shown to upregulate CART peptide expression in stress-regulatory regions of the brain, the hypothalamus and the amygdala (Balkan et al., 2006; Gozen et al., 2007). Forced swim has long-term (24 hours after the forced swim) effects on stress related peptides and mRNAs (Veenema et al. 2003; Gesing et al. 2001). Additionally, transcription of significant amounts of specific mRNAs can occur within 5 to 20 minutes (Ben-Ari et al. 2010; Femino et al. 1998). Furthermore, forced swim is reported to induce CRH transcription immediately after 10 minutes swimming (Jiang et al. 2004). Therefore, we selected the 2-day protocol to observe both the

long-term (24 hours after the first swim) and the short-term (10 minutes after the second swim) effects on CART mRNA expression.

4.4. In situ hybridization

Rats were sacrificed by decapitation between 09:00 and 11:00 (stressed groups: 10 min after the final stress exposure on the second day of forced swim). All tissues were stored at −80°C until assayed. Fourteen-micrometer thick coronal sections of the brains through the PVN, DMH and ARC nuclei were cut in a freezing microtome and mounted directly on microscope slides. Slides were stored at −80°C until used.

The protocol for in situ hybridization follows that described byBalkan et al. (2003), Hunter et al. (2005, 2006) and Jones and Kuhar (2006). An oligonucleotide probe complementary to nucleotides 223–270 of rat CART mRNA was used. This oligonucleotide was synthesized by the Emory University Microchemical Facility. The oligonucleotide was labeled using ^{35}S-dATP (Perkin Elmer Life Sciences, Boston, MA) at the 3′ end with terminal deoxynucleotidyl transferase (Amersham Pharmacia Biotech, Piscataway, NJ) and purified using QIAquick Nucleotide Removal Kit (QIAGEN, Chatsworth, CA). The probe was heated at 80°C for 3 min. A 16% 50 × Tris-EDTA (pH 7.4) solution containing 0.01 M dithiothreitol and 0.4 μg/ml sonicated salmon sperm DNA (Amersham Pharmacia Biotech) was added to the probe. This probe solution was mixed into the hybridization solution containing 50% formamide, 10% dextran sulfate, 0.3 M NaCl, 1.6% 50 × TE solution (pH 7.4), 2% 50 × Denhardt's solution (Sigma), and 8 mM dithiothreitol. 180 μl of the final hybridization buffer contained 5–6 × 10^5 cpm labeled probe.

The frozen tissue sections were placed in a vacuum desiccator for 1 h, fixed in 4% paraformaldehyde for 5 min, rinsed in 1 × phosphate buffered saline (PBS) for 2 × 5 min, and treated with 0.5% acetic anhydride in 0.1 M triethanolamine for 10 min. After a 3-min wash in 2 × saline sodium citrate (SSC), sections were dehydrated in increasing concentrations of ethanol, dipped in chloroform, rinsed in EtOH, and air-dried. The prehybridization buffer, a hybridization buffer without the probe solution, was applied (150 μl/slide) on the tissue sections. The sections were covered and incubated at 42°C for 2 h. Following the incubation, sections were washed twice with 2 × SSC for 3 min, rinsed in EtOH, and air-dried. Hybridization buffer was applied (180 μl/slide) on the sections and after covering, sections were incubated at 42°C overnight.

The next day, sections were washed 4 × 15 min with 1x SSC at 55°C and allowed to cool at room temperature for 2 h. Then sections were washed in a solution containing 0.3 M ammonium acetate and 50% EtOH for 3 min, 0.3 M ammonium acetate and 85% EtOH for 3 min, and in 100% EtOH for 3 min. Sections were air-dried and co-exposed to X-ray films (BioMax MR film, Kodak, Rochester, NY) with autoradiographic ^{14}C-labeled radioactive standards (ranging from 0.063–2.15 μCi/g) for 6 days.

To ensure reliable comparison among different groups, one animal from each of the four experimental groups (male control, male stressed, female control, and female stressed) were included in each experimental set. There were 6 experimental sets. In situ hybridization procedures and autoradiographic film exposures were performed on the same days for the four animals in the same set.

4.5. Quantitative analysis

During data analysis, for each animal, 8 consecutive sections containing the PVN (rostral-caudal: −1.80 to −2.12 mm from bregma), 8 consecutive sections (rostral-caudal: −2.60 to −3.14 mm from bregma) containing the DMH and 16 sections (rostral-caudal: −2.60 to −3.30 mm from bregma) containing the ARC nucleus were selected according to the

coordinates defined in the stereotaxic atlas (Paxinos and Watson, 1998). Autoradiographic film images of the selected sections were analyzed on a computerized image analysis system (Image J, NIH, USA). Optical densities of the sections were measured by constructing third degree polynomial calibration curves using the ^{14}C-labeled radioactive standards. In every section, CART mRNA levels were quantified as the area of expression with a mean optical density above background (area × mean optical density = arbitrary units). Finally, for each hypothalamic nucleus in every animal, an average arbitrary unit of expression was calculated.

4.6. Statistical evaluation

The SPSS (v17.0) program was used for all statistical analyses. CART mRNA levels, determined as average arbitrary units, were calculated as % control of the same sex. One-sample t-tests were performed to see the variance from 100% (control) in the experimental groups. Independent samples t-tests were used to compare the differences between the male stressed and female stressed groups.

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Research Highlights

- Forced swim stress increased CART mRNA in female paraventricular nucleus.
- Forced swim stress did not change CART mRNA levels in male paraventricular nucleus.
- There was a significant sex difference in paraventricular CART mRNA levels.
- Forced swim stress increased CART mRNA in dorsomedial hypothalamus of both sexes.
- Forced swim stress decreased CART mRNA in ARC nucleus of both sexes.

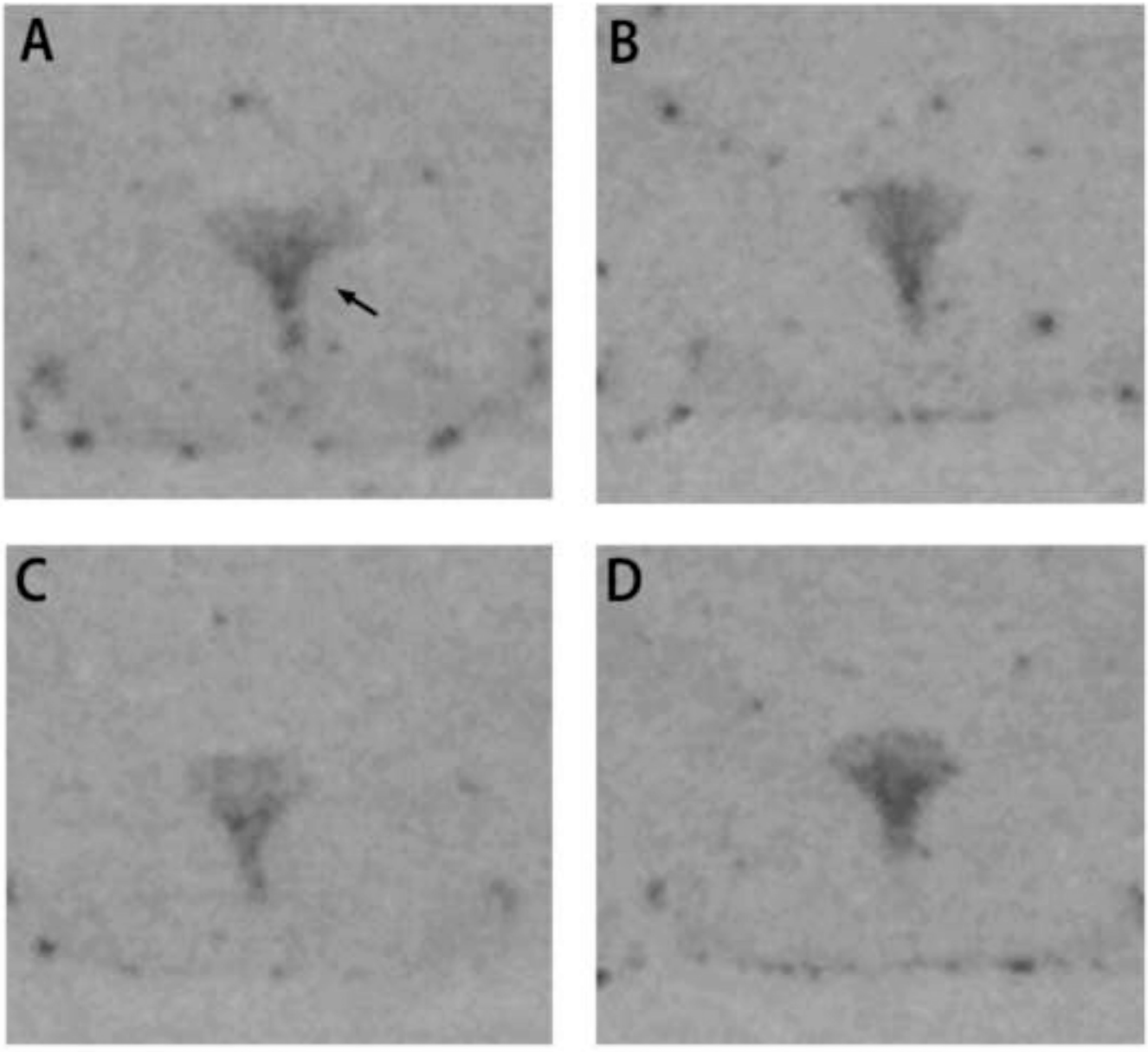

Figure 1.
Autoradiograms of CART mRNA in situ hybridization signal in the paraventricular nucleus (PVN, indicated by an arrow on the left upper image) of male control (A), male stressed (B), female control (C) and female stressed (D) rats. Forced swim stress increased CART mRNA levels significantly only in the female PVN. Typical examples are shown.

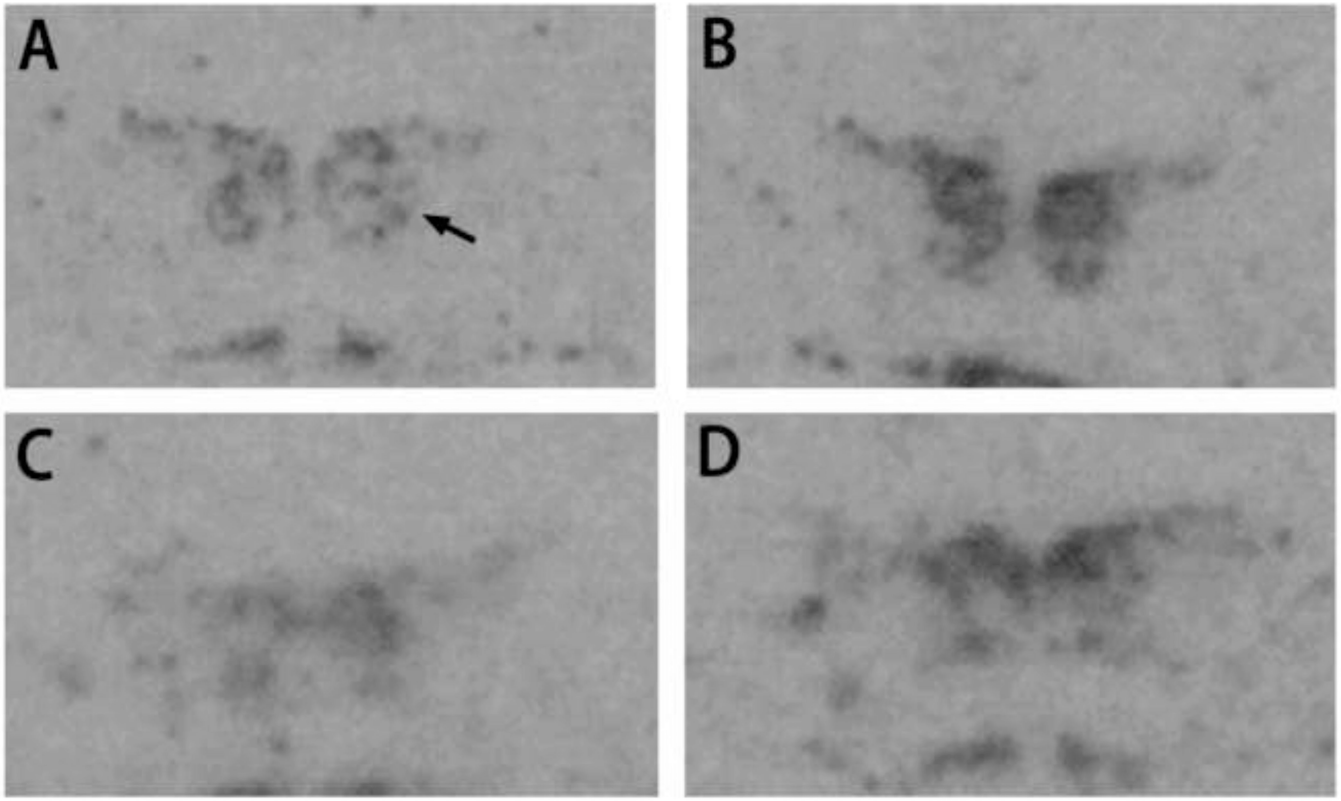

Figure 2.
Autoradiograms of CART mRNA in situ hybridization signal in the dorsomedial nucleus (DMH, indicated by an arrow on the left upper image) of male control (A), male stressed (B), female control (C) and female stressed (D) rats. Forced swim stress increased CART mRNA levels significantly both in the female and male DMH. Typical examples are shown.

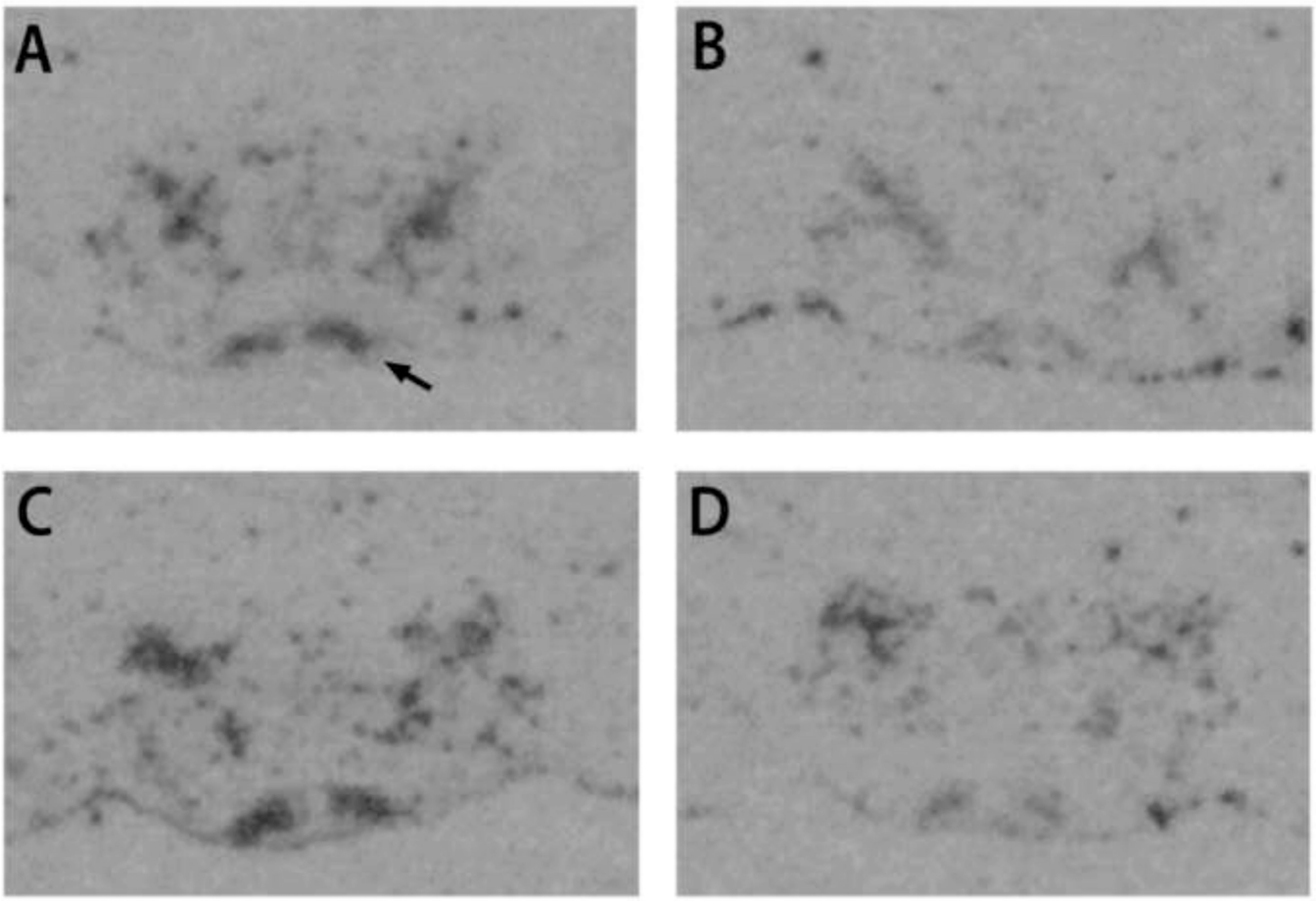

Figure 3.
Autoradiograms of CART mRNA in situ hybridization signal in the arcuate nucleus (ARC, indicated by an arrow on the left upper image) of male control (A), male stressed (B), female control (C) and female stressed (D) rats. Forced swim stress decreased CART mRNA levels significantly both in the female and male ARC. Typical examples are shown.

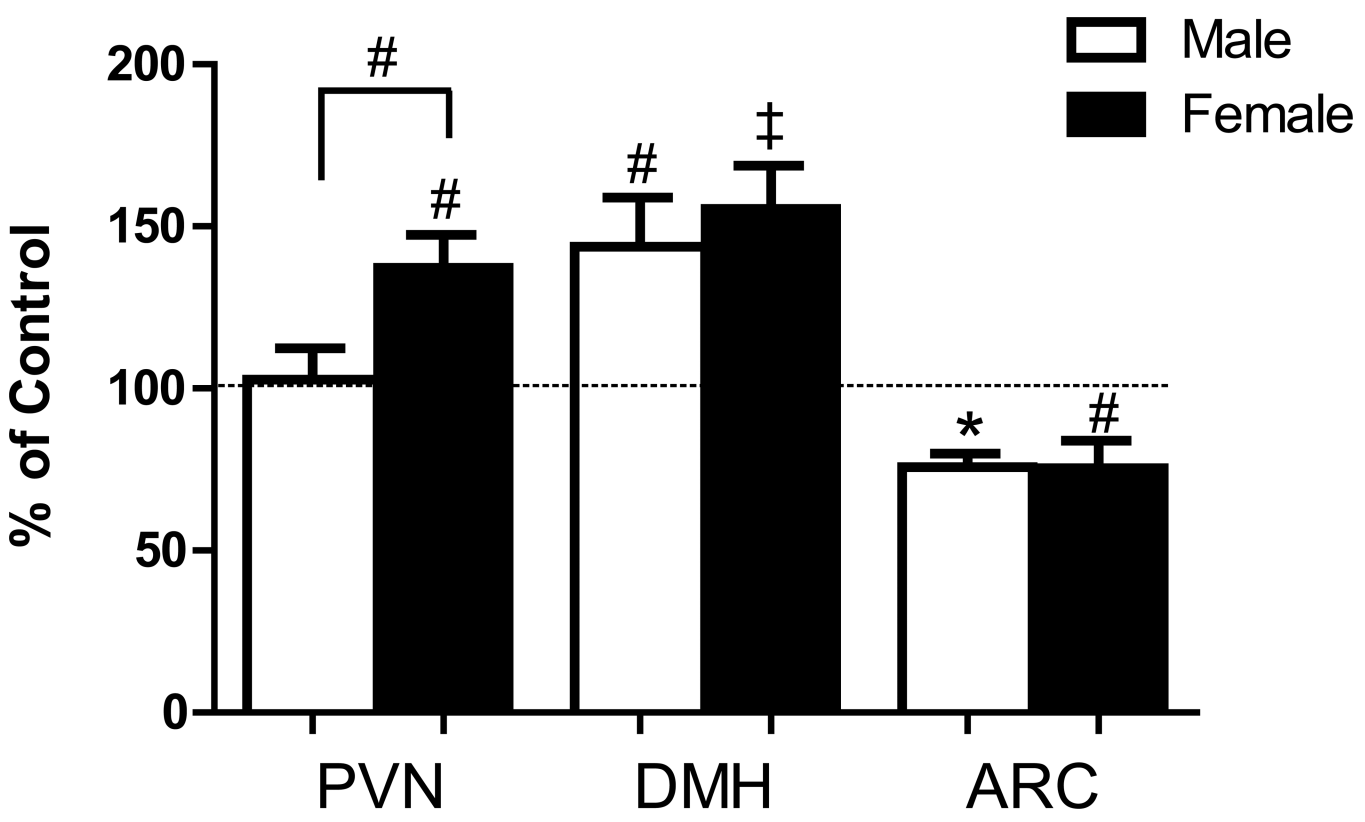

Figure 4.

CART mRNA levels in the paraventricular (PVN), dorsomedial (DMH) and arcuate (ARC) nuclei of the hypothalamus following forced swim stress. Forced swim stress upregulated CART mRNA levels in the PVN of female rats and in the DMH of both sexes. Forced swim stress downregulated CART mRNA levels in the ARC nucleus of both sexes. No effect was observed in the male PVN. The bars represent mean ± SEM arbitrary units expressed as % of values in control animals (n=6). Differences from control (100%): $^{*}p<0.0005$, $^{\#}p<0.05$, $^{‡}p<0.01$, one sample t-test. Difference from male: $^{\#}p<0.05$, independent samples t-test