ORIGINAL ARTICLE

# The influence of cocaine-amphetamine regulated transcript (CART) on pituitary hormones, corticosterone and leptin levels in starved rats

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**Abstract**

**OBJECTIVE:** CART is involved in the control of food intake and hormonal secretion. We aimed to evaluate the effects of CART on hormonal profile in starved rats.

**METHODS:** Study group included 100 male rats. Under conditions of food limitation CART (55–102) was given centrally (icv) or peripherally (iv). Non-starved animals underwent identical procedure. Vehicle (aCSF or saline)-injected rats served and as a controls. 60 minutes after CART or vehicle administration blood was collected to assess pituitary hormones (LH, FSH, PRL, GH, ACTH, TSH), corticosterone and leptin concentrations.

**RESULTS:** Itracerebroventricular CART injection resulted in a significant increase in PRL, GH and corticosterone concentrations in non-starved rats compared with vehicle injected animals. However, in a group of starved animals only leptin levels were decreased in comparison with fasted controls.

Peripheral CART administration caused a significant increase in PRL, GH and TSH levels in non-starved rats but no changes in investigated hormone levels were observed in starved animals when compared to saline injected controls.

**CONCLUSIONS:** Our results indicate that CART is able to modulate hormonal profile in a non-starved rats. However, the modulatory effect depends on the CART administration method. Interestingly, CART administration, both icv and iv, does not have an impact on pituitary hormones and corticosterone levels in a course of food limitation.

## INTRODUCTION

The cocaine and amphetamine-regulated transcript (CART) is a group of peptides widely expressed in the central nervous system (CNS), especially in the hypothalamus and pituitary, as well as in several other organs including the adrenals and pancreas (Koylu *et al.* 1997; Wierup *et al.* 2007).

CART is expressed in the hypothalamic nuclei which are involved in the control of food intake and hormonal secretion (Elias *et al.* 2001). Moreover, CART has also been identified in the gut, especially in the antral gastrin-producing G-cells and in vagal nerves (Wierup *et al.* 2007; Murphy

2005; Dockray 2009; de Lartigue *et al.* 2007). In rats, CART mRNA has two splice variants: one that encodes a long form of pro-CART and another that encodes a short form. The fragments of the long form of pro-CART comprise amino acids 55–102 and 62–102 (de Lartigue *et al.* 2007). These two active CART peptide fragments have been reported to affect the control of food consumption (Rogge *et al.* 2008; Thim *et al.* 1997) and locomotion activity (Kimmel *et al.* 2002).

Although several behavioral and biochemical studies have suggested the existence of multiple CART receptors, the exact location of these receptors is still not known, even though specific CART binding has been reported in the AtT20 pituitary cell line (Vicentic *et al.* 2006).

CART belongs to an important family of neuropeptides that are involved in many physiological functions. It has been suggested that it plays a pivotal role in the regulation of feeding behavior and metabolic activity (Rogge *et al.* 2008). Some studies have shown that icv administration of CART decreases food intake in rats (Rogge *et al.* 2008; Lambert *et al.* 1998). Moreover, CART also increases thermogenesis, energy expenditure and inhibits gastric emptying (Hunter *et al.* 2004). CART also mediates the effects of serotonin-4-receptor activation on food intake (Jean *et al.* 2007) and it colocalizes with melanocyte-stimulating hormone (MSH), an inhibitor of food intake in the arcuate nucleus (ARC) (Tian *et al.* 2004; Dhillo *et al.* 2002). Furthermore, CART is also involved in the orexigenic effects of anandamide that is an endogenous cannabinoid neurotransmitter (Osei-Hyiaman *et al.* 2005). These findings indicate an interaction between CART and the endocannabinoid system. Moreover, CART mRNA levels in the ARC were found to be increased by the administration of leptin, and leptin receptors were identified on CART-containing cells in the ARC (Wang *et al.* 1999). It has been speculated that hypothalamic CART mRNA levels are regulated by leptin. Fasting and a decrease in circulating leptin result in a reduction in CART mRNA expression (Murphy 2005). These data suggest that CART is a mediator of leptin effects.

The colocalization and interaction of CART with both orexigenic and anorexigenic neuropeptides indicate that CART may play a modulatory role in feeding behavior (Hunter *et al.* 2004). In addition, CART interacts with many other mechanisms that are involved in feeding and energy expenditure (Rogge *et al.* 2008).

CART is also an important factor in a stress reaction, probably through its effect on corticotropin-releasing hormone (CRH), and modulation of immunological activity (Kuhar et al. 2002; Bik *et al.* 2008).

Besides, CART may also influence the release of other hypothalamic neuropeptides. It has been shown that CART mRNA colocalizes with the transcripts for vasopressin, thyrotropin-releasing hormone (TRH) and CRH in the paraventricular nucleus (PVN) (Kuhar *et al.* 2002; Li *et al.* 2002). It has also been reported that CART can stimulate CRH, TRH and NPY release from hypothalamic explants *in vitro* (Stanley *et al.* 2001).

The processes of food intake are under control of several neurohormonal mechanisms. The hypothalamus and its nuclei are the sites where the hunger and satiety centers are located. Besides, ARC and PVN are thought to be the sites where signals converge to regulate food intake and energy expenditure (Konturek *et al.* 2005). Immunohistochemical studies have identified the presence of CART in the hypothalamus, pituitary and adrenal glands (Koylu *et al.* 1997) as well as in portal blood including the hypophysial-portal circulation (Larsen *et al.* 2003). These findings indicate that CART influences the regulation of hormone secretion, although the mechanism by which it does this is uncharacterized and controversial. Despite the presence and activity of CART peptides in the hypothalamic regions involved in the appetite control, to date the role of CART in the regulation of hormone secretion in starvation has not been determined.

The aim of this study was to compare the effects of CART on pituitary hormones, corticosterone and leptin concentrations in starved and non-starved rats.

## MATERIALS AND METHODS

*Animals*

Male Wistar rats three-month-old (weight approx. 240–260 g) were maintained under controlled conditions (12 h L:12 h D, lights on at 06:00 h, temperature at 22±1°C). The total number of animals was one hundred. On the day of experiment the animals were assigned to the subgroups according to the procedure scheme.

All experimental procedures were approved by the First Warsaw Ethics Committee for Experiments on Animals (M. Nencki Institute of Experimental Biology, the Polish Academy of Sciences).

*Experiment 1: Intracerebroventricular (icv) administration of CART*

Forty animals were assigned for the experiment. The animals were anesthetized intramuscularly with ketamine, xylazine and atropine, and a 23-gauge stainless steel guide cannula was implanted in the third cerebroventricle (0.8 mm posterior and 7.0 mm ventral to the bregma at the midline) according to the atlas of Paxinos & Watson (1986). The cannula was closed with a removable stainless-steel plug. After surgery the rats were transferred to individual cages with food and water freely available. Over a 7-day period of recovery, rats were handled daily to minimize any stress associated with handling during the experiment. After the recovery period the animals were randomly divided into two groups of starved (for 72 hours) or non-starved rats (20 rats in each group). Both groups had free access to water. Thereafter, on the day of the experiment, 2 h before CART administration, the stainless-steel guide

cannula was opened and its patency confirmed. Intracerebroventricular infusion of CART (55–102) (rat; Bachem) was performed on freely moving rats. Using an automatic pump (CMA/100; Sweden), 0.5 µg of CART in 5 µl vehicle (artificial cerebrospinal fluid, aCSF), or the same volume of vehicle alone, was slowly (1 µl/min) infused into the third ventricle through an inner cannula inserted into the guide cannula. After the infusion the rats were transferred to their home cages with free access to water. At 60 min after the infusion of CART or vehicle, animals were decapitated and trunk blood was collected in plastic tubes containing 500 IU aprotinine (inhibitor of protease; Trascolan) per ml of blood. The time from removal of the animals from their cages to blood collection was approximately 2 min. At the end of the experiment the placement of the intracerebroventricular cannula was verified by injection of trypan blue dye. The brain was inspected to verify the spread of the marker dye throughout the third ventricle. Data from any animal that showed an inadequate spread of the dye were discarded. The blood samples were centrifuged (2 000 rpm for 20 min at 4°C). Serum was collected and stored at –70°C until hormone analyses were performed.

*Experiment 2: Intravenous (iv) injection of CART*

The experiment was carried out on 20 starved (for 72 hours) and 20 fed ad libitum rats. A dose of 10 µg CART (55–102) in 300 µl of saline (0.9% NaCl) or 300 µl of saline alone was injected into the tail vein. The animals were then transferred to individual cages. At 60 min after the injection of CART or saline, the animals were decapitated and trunk blood was collected in plastic tubes containing 500 IU of aprotinine per ml of blood. The blood samples were centrifuged (2000 rpm for 20 min at 4 °C) and serum was stored at –70 °C until hormone analyses were performed.

*Hormone analyses*

Concentrations of LH, FSH, PRL and TSH in samples of serum were measured by RIA using reagents prepared by Dr A.F. Parlow and provided by the NIDDK (Bethesda, MD, USA). Values were expressed in relation to LH-RP-3, FSH-RP-2, PRL-RP-3 and TSH-RP-3 reference standards, respectively. The limit of detection varied for the individual hormones: LH – 0.1 ng/ml, FSH – 1.25 ng/ml, PRL – 0.39 ng/ml and TSH – 0.3 ng/ml.

Concentrations of rLeptin and rGH were measured using a RIA kit from Linco Research (Linco Research, St. Charles, MO, USA.) The sensitivity of both assays was 0.5 ng/ml. Concentrations of ACTH were determinated using a commercial kit from Phoenix USA (Phoenix Pharmaceuticals, Inc, Burlingame, CA, USA). The detection limit for this assay was 10 pg/ml.

Serum corticosterone concentrations were measured using a RIA kit from BM Biomedical Structures, LLC (Biomedical Structures LLC, Warwick RI, USA). The limit of detection for corticosterone was 25 ng/ml. All measurements were made in duplicate in one assay. Intra-assay coefficients of variation (CV) were <7%.

*Statistical analysis*

All results are expressed as mean ±SD. Statistical evaluation of differences between groups was performed using the Kruskal-Wallis rank test followed by the Mann-Whitney U-test. Results were considered statistically significant when $p<0.05$.

## RESULTS

The results are presented in Tables 1, 2, 3 and 4.

*ICV procedure*

Intracerebroventricular administration of CART caused a significant increase in serum concentration of PRL, GH and corticosterone ($p<0.01$, $p<0.05$, $p<0.01$, respectively) in non starved rats compared with vehicle injected animals. However, in a group of starved animals only levels of leptin were found to be decreased ($p<0.01$) in comparison with CSF-treated rats. CART treatment failed to produce any significant changes in other examined hormones levels in this group of rats (Table 1).

Fasting influenced hormone release in the animals treated only with CSF. Increase of corticosterone levels ($p<0.01$) was noticed in the group of starved animals. Suppression of gonadotropins (LH and FSH), GH and leptin concentration ($p<0.05$, $p<0.05$, $p<0.05$, $p<0.01$ respectively) was noticed in these rats under conditions of food limitation (Table 2).

Comparison between non-starved and starved rats treated icv with injection of CART revealed that in starved animals values of ACTH, LH, FSH, PRL, GH, TSH and leptin were markedly decreased ($p<0.01$ for all examined pituitary hormones and $p<0.001$ for leptin, respectively) (Table 2).

*IV procedure*

Intravenous injection of CART resulted in a significant increase in PRL, GH and TSH levels in non-starved rats ($p<0.05$, $p<0.05$, $p<0.05$, respectively), but no changes in hormone levels were observed in starved animals when compared to saline injected controls (Table 3).

Comparison between saline-injected animals revealed that in fasted animals levels of ACTH, LH, PRL, GH, TSH and leptin were significantly decreased ($p<0.001$, $p<0.05$, $p<0.01$, $p<0.01$, $p<0.01$, $p<0.05$ and $p<0.001$, respectively) and values of corticosterone were enhanced ($p<0.05$) (Table 4).

Moreover, significant differences in concentrations of FSH, PRL, GH and TSH as well as those of leptin were seen between CART-treated non-starved and starved rats with lower values found in the fasted group ($p<0.05$, $p<0.01$, $p<0.01$, $p<0.001$ and $p<0.001$, respectively) (Table 4).

## DISCUSSION

The relationship between starvation and hormonal disturbances has been investigated for many years. It has been reported that fasting leads to activation of the hypothalamic-pituitary-adrenal axis and to suppression of the gonadal, somatotropic and thyroid axes both in humans and rats (Lawson & Klibanski 2008; Kmiec *et al.* 2006). The changes in hormone secretion are thought to be connected with the altered activity of many peptides like NPY, leptin and galanin that are involved in the regulation of feeding behavior and hormone release (Baranowska *et al.* 2001). Moreover, the impairment of reproductive function can also occur under conditions of food restriction and/or increased energy expenditure (Aubert & Sizonenko 1996). Previously, we found that the hormone release response following administration of peptides that participate in the regulation of appetite such as leptin, NPY and galanin is impaired in starved rats (Baranowska *et al.* 2001).

In the present study, as expected, we found several differences in investigated hormone concentrations between groups of non-starved and starved rats. Furthermore, CART-treatment influenced hormonal status during fasting. Interestingly, the method of CART administration had also impact on hormone concentrations. Thus, the results of our study reveal that the hormonal response to the administration of CART (55–102) is markedly altered in starved rats.

As it has been mentioned above, starvation results in a deprivation of the gonadal axis. Kuriyama *et al.* (2004) demonstrated the colocalization of CART with FSH and LH in the same secretory granules of gonadotroph cells. It has been reported in the literature that CART stimulates pulsatile GnRH secretion from hypothalamus (Lebrethon *et al.* 2000; Parent *et al.* 2000). We found the

**Tab. 1.** Serum pituitary hormones, corticosterone and leptin concentrations at 60 min after icv administration of CART or vehicle in non-starved and starved rats.

| | Non-starved rats | | | Starved rats | | |
|---|---|---|---|---|---|---|
| | $C_1$ | p-value | CART | $C_2$ | p-value | CART |
| **ACTH pg/ml** | 271 ± 57 | ns | 270 ± 24 | 228 ± 58 | ns | 195 ± 42 |
| **LH ng/ml** | 0.52 ± 0.30 | ns | 0.32 ± 0.12 | 0.21 ± 0.04 | ns | 0.18 ± 0.02 |
| **FSH ng/ml** | 5.9 ± 1.9 | ns | 6.0 ± 1.25 | 4.4 ±1.2 | ns | 3.9 ± 1.0 |
| **PRL ng/ml** | 3.8 ± 1.7 | <0.01 | 11 ± 4.3 | 3.5 ± 1.0 | ns | 3.2 ± 1.2 |
| **GH ng/ml** | 4.8 ± 2.5 | <0.05 | 9.0 ± 2.4 | 2.2 ± 1.39 | ns | 1.8 ± 1.1 |
| **TSH ng/ml** | 1.3 ± 0.70 | ns | 1.5 ± 0.60 | 1.0 ± 0.30 | ns | 0.86 ± 0.25 |
| **Corticosterone ng/ml** | 125 ± 73 | <0.01 | 500 ± 219 | 382 ± 267 | ns | 546 ± 204 |
| **Leptin ng/ml** | 4.9 ± 1.8 | ns | 6.8 ± 2.0 | 2.3 ± 0.85 | <0.05 | 1.3 ± 0.70 |

Data are presented as mean ± SD
$C_1$ – vehicle (artificial cerebrospinal fluid) injected non-starved rats
$C_2$ - vehicle (artificial cerebrospinal fluid) injected starved rats

**Tab. 2.** The comparison of serum pituitary hormones, corticosterone and leptin concentration between non-starved and starved rats treated with icv injection of vehicle or CART (60 min after administration).

| | Non-starved vs. Starved rats | | | Non-starved vs. Starved rats | | |
|---|---|---|---|---|---|---|
| | $C_1$ | *p*-value | $C_2$ | CART | *p*-value | CART |
| **ACTH pg/ml** | 271 ± 57 | ns | 228 ± 58 | 270 ± 24 | <0.01 | 195 ± 42 |
| **LH ng/ml** | 0.52 ± 0.30 | <0.05 | 0.21 ± 0.04 | 0.32 ± 0.12 | <0.01 | 0.18 ± 0.02 |
| **FSH ng/ml** | 5.9 ± 1.9 | <0.05 | 4.4 ±1.2 | 6.0 ± 1.25 | <0.01 | 3.9 ± 1.0 |
| **PRL ng/ml** | 3.8 ± 1.7 | ns | 3.5 ± 1.0 | 11 ± 4.3 | <0.01 | 3.2 ± 1.2 |
| **GH ng/ml** | 4.8 ± 2.5 | <0.05 | 2.2 ± 1.39 | 9.0 ± 2.4 | <0.01 | 1.8 ± 1.1 |
| **TSH ng/ml** | 1.3 ± 0.70 | ns | 1.0 ± 0.30 | 1.5 ± 0.60 | <0.01 | 0.86 ± 0.25 |
| **Corticosterone ng/ml** | 125 ± 73 | <0.01 | 382 ± 267 | 500 ± 219 | ns | 546 ± 204 |
| **Leptin ng/ml** | 4.9 ± 1.8 | <0.01 | 2.3 ± 0.85 | 6.8 ± 2.0 | <0.001 | 1.3 ± 0.70 |

Data are presented as mean ± SD
$C_1$ – vehicle (artificial cerebrospinal fluid) injected non-starved rats
$C_2$ - vehicle (artificial cerebrospinal fluid) injected starved rats

decrease of both gonadotropins, LH and FSH, in starved rats injected with aCSF, but only LH was suppressed in fasted animals when vehicle (saline) was given intravenously. However, no changes in gonadotropins levels where observed due to the CART treatment when compared vehicle injected controls with investigated groups of non-starved and starved rats. Furthermore, a comparison between CART-treated animals divided in accordance with their feeding status revealed a significant decrease in LH and FSH concentrations in a group of starved rats when CART was given intracerebroventricularly, and a decrease in FSH values in fasted group in case of intravenous injection. The data concerning the relationship between CART and gonadotropin levels in fasting are lacking. The observed low gonadotropin levels and lack of response of LH and FSH secretion to CART injections, both icv and iv, in starved rats may be connected with a decrease in leptin levels. It has been reported that in *in vitro* experiments, leptin stimulated the release of GnRH from the medial basal hypothalamus, and gonadotropins from the dispersed anterior pituitary cells (Yu *et al.* 1997). Thus, leptin is also able to modulate hypothalamo-pituitary-gonadal axis.

Leptin has also been identified as the hub for the interaction between central and peripheral signals in the control of energy homeostasis. It has been reported that leptin receptors are expressed in the hypothalamus and leptin may modulate the activity of peptides controlling feeding behavior (Sahu 2003). Our present results indicate that starvation leads to inhibition of leptin secretion. This observation is in accordance with our previous study in which we demonstrated the inhibition of leptin, estradiol and progesterone release in starved female rats (Baranowska *et al.* 2001).

It has been reported that leptin may influence the expression of CART mRNA (Couceyro *et al.* 1997) and

**Tab. 3.** Serum pituitary hormones, corticosterone and leptin concentrations at 60 min after iv administration of CART or vehicle in non-starved and starved rats.

| | Non-starved rats | | | Starved rats | | |
|---|---|---|---|---|---|---|
| | $C_3$ | *p*-value | CART | $C_4$ | *p*-value | CART |
| ACTH pg/ml | 202 ± 33 | ns | 189 ± 71 | 138 ± 16 | ns | 131 ± 19 |
| LH ng/ml | 0.40 ± 0.31 | ns | 0.40 ± 0.30 | 0.17 ± 0.04 | ns | 0.22 ± 0.07 |
| FSH ng/ml | 6.7 ± 0.75 | ns | 6.9 ± 1.2 | 5.9 ± 0.80 | ns | 5.8 ± 0.80 |
| PRL ng/ml | 5.8 ± 1.8 | <0.05 | 14 ± 11 | 3.0 ± 0.96 | ns | 4.4 ± 2.0 |
| GH ng/ml | 4.5 ± 1.2 | <0.01 | 25 ± 11.5 | 2.1 ± 1.2 | ns | 3.5 ± 2.6 |
| TSH ng/ml | 1.0 ± 0.20 | <0.01 | 1.7 ± 0.20 | 0.70 ± 0.10 | ns | 0.80 ± 0.20 |
| Corticosterone ng/ml | 186 ± 71 | ns | 189 ± 63 | 312 ±130 | ns | 210 ± 122 |
| Leptin ng/ml | 5.6 ± 1.5 | ns | 5.3 ± 1.3 | 0.43 ± 0.11 | ns | 0.45 ± 0.12 |

Data are presented as mean ± SD
$C_3$ – vehicle (0.9% NaCl) injected non-starved rats
$C_4$ - vehicle (0.9% NaCl) injected starved rats

**Tab. 4.** The comparison of serum pituitary hormones, corticosterone and leptin concentration between non-starved and starved rats treated with iv injection of vehicle or CART (60 minutes after administration).

| | Non-starved vs. Starved rats | | | Non-starved vs. Starved rats | | |
|---|---|---|---|---|---|---|
| | $C_3$ | *p*-value | $C_4$ | CART | *p*-value | CART |
| ACTH pg/ml | 202 ± 33 | <0.001 | 138 ± 16 | 189 ± 71 | ns | 131 ± 19 |
| LH ng/ml | 0.40 ± 0.31 | <0.05 | 0.17 ± 0.04 | 0.40 ± 0.30 | ns | 0.22 ± 0.07 |
| FSH ng/ml | 6.7 ± 0.75 | ns | 5.9 ± 0.80 | 6.9 ± 1.2 | <0.05 | 5.8 ± 0.80 |
| PRL ng/ml | 5.8 ± 1.8 | <0.01 | 3.0 ± 0.96 | 14 ± 11 | <0.01 | 4.4 ± 2.0 |
| GH ng/ml | 4.5 ± 1.2 | <0.01 | 2.1 ± 1.2 | 25 ± 11.5 | <0.01 | 3.5 ± 2.6 |
| TSH ng/ml | 1.0 ± 0.20 | <0.01 | 0.70 ± 0.10 | 1.7 ± 0.20 | <0.001 | 0.80 ± 0.20 |
| Corticosterone ng/ml | 186 ± 71 | <0.05 | 312 ±130 | 189 ± 63 | ns | 210 ± 122 |
| Leptin ng/ml | 5.6 ± 1.5 | <0.001 | 0.43 ± 0.11 | 5.3 ± 1.3 | <0.001 | 0.45 ± 0.12 |

Data are presented as mean ± SD
$C_3$ – vehicle (0.9% NaCl) injected non-starved rats
$C_4$ - vehicle (0.9% NaCl) injected starved rats

during starvation the expression of CART in ARC is lowered (Van Vugt *et al.* 2006). Besides, in our present study no significant differences in leptin concentrations were found when compared vehicle injected intravenously and CART treated animals independently of their feeding status. The comparison between leptin levels in rats injected intracerebrovenrticularly with vehicle or CART indicated that only in starved animals CART treatment decreased serum leptin values.

The results from our study confirm that starvation modulates secretion of prolactin. Assessment of prolactin concentrations in response to food restriction revealed that in saline injected animals the values of prolactin were lower in starved rats in comparison with those fed ad libitum and no changes were found in animals that were given aCSF. Furthermore, increased PRL release in response to icv and iv injection of CART was observed in non-starved rats. In addition, intracerebroventricular CART treatment resulted in suppression of PRL levels in starved rats as lower values of this hormone were found in comparison with equivalent non-starved rats. CART may influence PRL secretion in multiple ways. However, the mechanism underlying the regulation of PRL release by CART remains unclear and controversial (Baranowska *et al.* 2007). The stimulatory effect of CART on PRL release in non-fasted animals may be a result of inhibition of hypothalamic dopamine (Brunetti *et al.* 2000), stimulation of hypothalamic TRH (Stanley *et al.* 2001), and/or a direct effect on the pituitary (Kuriyama *et al.* 2004).

TSH levels may also be altered in starvation. However, in our study concentrations of TSH did not differ between icv treated starved and non-starved controls, but iv injection of vehicle resulted in decrease in TSH levels in starved controls when compared with non-starved ones. Serum TSH values were affected by CART iv administration only in non-starved animals. It has been reported that CART may be contained in the fibers that modulate TRH gene expression and the biosynthesis of TRH (Fekete *et al.* 2000). CART is also co-expressed with TRH in the hypothalamic PVN (Broberger 1999). In addition, CART is synthesized in hypophysiotropic TRH neurons and co-released with TRH into the pituitary circulation (Fekete *et al.* 2000).

The increased GH release observed in non-starved rats after CART injection, both icv and iv, may be a result of the stimulatory influence of CART on GH-RH, TRH or an inhibitory effect on the somatostatinergic system. In control starved rats we observed a decrease in GH release when compared results with those of equivalent non-starved rats and no changes in GH levels were found following icv or iv injections of CART. Results obtained in non-starved group are in agreement with our previous studies (Baranowska *et al.* 2004). To date, no studies on influence of CART on GH secretion during fasting have been published.

Starvation affects the hypothalamo-pituitary-adrenal axis (Kmiec *et al.* 2006). This statement was confirmed in our study as an inhibition of ACTH release and increase of corticosterone concentrations were noticed in control (saline injected iv) starved rats compared with equivalent non-starved animals. Interestingly, in case of icv administration of vehicle no differences in ACTH values were noticed but enhance in corticosterone concentration was seen in fasted control rats. However, CART administered iv did not significantly change these concentrations but infused icv caused a significant suppression of ACTH but not corticosterone in fasted animals in comparison with those without food limitation.

Data from our already published study suggests that effect of CART, administered centrally, on the pituitary-adrenal axis in non-starved rats is short lasting (Baranowska *et al.* 2006). Briefly, in that particular experiment only a short stimulatory effect of CART on ACTH was detected (only at 30 min after CART icv injection). However, stimulation of corticosterone levels was observed at both 30 and 60 min (Baranowska *et al.* 2006). All these results corroborate the findings of others. Stanley *et al.* (2001) also observed a significant increase of serum ACTH shortly after the administration of CART. Vrang *et al.* (2000) have suggested that CART may activate central CRH neurons and oxytocinergic neurons of the paraventricular nucleus.

A number of pieces of evidence support the suggestion that CART is able to activate the CRH-ACTH axis. In details, CART-IR colocalizes with neuropeptides known to modulate the hypothalamo-pituitary-adrenal axis. CART is present in the PVN, which contains CRH-expressing neurons (Ceccatelli *et al.* 1989). Lastly, Vrang *et al.* (2000) demonstrated that icv injection of CART (55–102) increases the expression of the early gene, c-fos, in CRH- containing PVN neurons.

However, the short-term stimulation of ACTH release and more prolonged stimulation of corticosterone release that we detected in our previous study after icv CART injection suggest that, in addition to stimulation via CRH-ACTH (Smith *et al.* 2004), CART may also exert a direct effect on the adrenals (Baranowska *et al.* 2006).

Fasting results in disturbances in the activity of many peptides involved in the control of energy homeostasis and hormone secretion. The permeability of the blood-brain barrier and the secretory ability of pituitary cells may also be altered in starvation. The varied and sometimes opposite responses of hormone release to CART in starvation confirms the hypothesis that CART has an important role in the regulation of energy homeostasis and hormone secretion.

It is also worth noticing that the present study has for the first time demonstrated the wide spectrum of effects of CART on hormone release in starved rats.

## CONCLUSIONS

1. Starvation leads to disturbances in the release of leptin, pituitary and adrenal hormones.
2. The modulatory effect of CART on the activity of pituitary and adrenal hormones is observed both after central and peripheral administration of CART
3. Hormone release in response to CART administration is altered in starved rats.

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REFERENCES

1 Aubert ML, Sizonenko PC (1996). Environmental factors and sexual maturation in rodents. Acta Paediatr Suppl. **417:** 86–88.
2 Baranowska B, Wasilewska-Dziubińska E, Radzikowska M, Płonowski A, Roguski K (1997). Neuropeptide Y, galanin, and leptin release in obese women and in women with anorexia nervosa. Metabolism. **46:** 1384–1389.
3 Baranowska B, Chmielowska M, Wolińska-Witort E, Roguski K, Wasilewska-Dziubińska E (2001). The relationship between neuropeptides and hormones in starvation. Neuro Endocrinol Lett. **22:** 349–355.
4 Baranowska B, Wolińska-Witort E, Martyńska L, Chmielowska M, Baranowska-Bik A (2004). Effects of cocaine-amphetamine regulated transcript (CART) on hormone release. Regul Pept. **122:** 55–59.
5 Baranowska B, Wolińska-Witort E, Bik W, Baranowska-Bik A, Martyńska L, Chmielowska M (2006). The effect of cocaine-amphetamine regulated transcript (CART) on the activation of the pituitary-adrenal axis. Neuro Endocrinol Lett. **27:** 60–62.
6 Baranowska B, Baranowska-Bik A, Bik W, Wolińska-Witort E, Martyńska L, Chmielowska M (2007). Controversial opinions on the role of cocaine and amphetamine-regulated transcript (CART) in prolactin release. Neuro Endocrinol Lett. **28:** 541–544.
7 Bik W, Skwarło-Sońta K, Szelągiewicz J, Wolińska-Witort E, Chmielowska M, Martyńska L et al (2008). Involvement of the cocaine-amphetamine regulated transcript peptide (CART 55–102) in the modulation of rat immune cell activity. Neuro Endocrinol Lett. **29:** 359–365.
8 Broberger C (1999). Hypothalamic cocaine- and ampethamine-regulated transcript (CART) neurons: histochemical relationship to thyrotropin-releasing hormone, melanin-concentrating hormone, orexin/hypocretin and neuropeptide Y. Brain Res. **848:** 101–113.
9 Brunetti L, Orlando G, Michelotto B, Recinella L, Vacca M (2000). Cocaine- and amphetamine-regulated transcript peptide-(55–102) and thyrotropin releasing hormone inhibit hypothalamic dopamine release. Eur J Pharmacol. **409:** 103–107.
10 Ceccatelli S, Eriksson M, Hökfelt T (1989). Distribution and coexistence of corticotropin-releasing factor-, neurotensin-, enkephalin-, cholecystokinin-, galanin- and vasoactive intestinal polypeptide/peptide histidine isoleucine-like peptides in the parvocellular part of the paraventricular nucleus. Neuroendocrinology. **49:** 309–323.
11 Couceyro PR, Koylu EO, Kuhar MJ (1997). Further studies on the anatomical distribution of CART by in situ hybridization. J Chem Neuroanat. **12:** 229–241.
12 de Lartigue G, Dimaline R, Varro A, Dockray GJ (2007). Cocaine- and amphetamine-regulated transcript: stimulation of expression in rat vagal afferent neurons by cholecystokinin and suppression by ghrelin. J Neurosci. **27:** 2876–2882.
13 Dhillo WS, Small CJ, Stanley SA, Jethwa PH, Seal LJ, Murphy KG et al (2002). Hypothalamic interactions between neuropeptide Y, agouti-related protein, cocaine- and amphetamine-regulated transcript and alpha-melanocyte-stimulating hormone *in vitro* in male rats. J Neuroendocrinol. **14:** 725–730.
14 Dockray GJ (2009). Cholecystokinin and gut-brain signalling. Regul Pept. **155:** 6–10.
15 Elias CF, Lee CE, Kelly JF, Ahima RS, Kuhar M, Saper CB, et al (2001). Characterization of CART neurons in rat and human hypothalamus. J Comp Neurol. **26:** 1–19.
16 Fekete C, Mihály E, Luo L-G, Kelly J, Clausen JT, Mao Q et al (2000). Association of cocaine- and amphetamine-regulated transcript-immunoreactive elements with thyrotropin-releasing hormone-synthesizing neurons in the hypothalamic paraventricular nucleus and its role in the regulation of the hypothalamic–pituitary–thyroid axis during fasting. J Neurosci. **20:** 9224–9234.
17 Hunter RG, Philpot K, Vicentic A, Dominguez G, Hubert GW, Kuhar MJ (2004). CART in feeding and obesity. Trends Endocrinol Metab. **15:** 454–459.
18 Jean A, Conductier G, Manrique C, Bouras C, Berta P, Hen R, et al (2007). Anorexia induced by activation of serotonin 5-HT4 receptors is mediated by increases in CART in the nucleus accumbens. Proc Natl Acad Sci USA. **104:** 16335–16340.
19 Kimmel HL, Thim L, Kuhar MJ (2002). Activity of various CART peptides in changing locomotor activity in the rat. Neuropeptides. **36:** 9–12.
20 Kmiec Z, Pokrywka L, Kotlarz G, Mysliwski A (2006). The effects of fasting and refeeding on adrenal cortex morphometry and serum concentrations of ACTH and corticosterone in young and old male rats. J Physiol Pharmacol. **57:** 77–84.
21 Konturek PC, Konturek JW, Cześnikiewicz-Guzik M, Brzozowski T, Sito E, Konturek SJ (2005). Neuro-hormonal control of food intake: basic mechanisms and clinical implications. J Physiol Pharmacol. **56:** 5–25.
22 Koylu EO, Couceyro PR, Lambert PD, Ling NC, DeSouza EB, Kuhar MJ (1997). Immunohistochemical localization of novel CART peptides in rat hypothalamus, pituitary and adrenal gland. J Neuroendocrinol. **9:** 823–833.
23 Kuhar MJ, Adams S, Dominguez G, Jaworski J, Balkan B (2002). CART peptides. Neuropeptides. **36:** 1–8.
24 Kuriyama G, Takekoshi S, Tojo K, Nakai Y, Kuhar MJ, Osamura RY (2004). Cocaine- and amphetamine regulated transcript peptide in the rat anterior pituitary gland is localized in gonadotrophs and suppresses prolactin secretion. Endocrinology. **145:** 2542–2550.
25 Lambert PD, Couceyro P, McGirr KM, Dall Vechia SE, Smith Y, Kuhar MJ (1998). CART peptides in the central control of feeding and interactions with neuropeptide Y. Synapse. **29:** 293–298.
26 Larsen PJ, Seier V, Fink-Jensen A, Holst JJ, Warberg J, Vrang N (2003). Cocaine- and amphetamine regulated transcript is present in hypothalamic neuroendocrine neurones and is released to the hypothalamic-pituitary portal circuit. J Neuroendocrinol. **15:** 219–226.
27 Lawson EA, Klibanski A (2008). Endocrine abnormalities in anorexia nervosa. Nat Clin Pract Endocrinol Metab. **4:** 407–414.
28 Lebrethon MC, Vandersmissen E, Gérard A, Parent AS, Bourguignon JP (2000). Cocaine and amphetamine-regulated-transcript peptide mediation of leptin stimulatory effect on the rat gonadotropin-releasing hormone pulse generator *in vitro*. J Neuroendocrinol. **12:** 383–385.
29 Li HY, Hwang HW, Hu YH (2002). Functional characterizations of cocaine- and amphetamine-regulated transcript mRNA expression in rat hypothalamus. Neurosci Lett. **323:** 203–206.
30 Murphy KG (2005). Dissecting the role of cocaine- and amphetamine-regulated transcript (CART) in the control of appetite. Brief Funct Genomic Proteomic. **4:** 95–111.
31 Osei-Hyiaman D, Depetrillo M, Harvey-White J, Bannon AW, Cravatt BF, Kuhar MJ et al (2005). Cocaine- and amphetamine related transcript is involved in the orexigenic effect of endogenous anandamide. Neuroendocrinology. **81:** 273–282.

32 Parent AS, Lebrethon MC, Gérard A, Vandersmissen E, Bourguignon JP (2000). Leptin effects on pulsatile gonadotropin releasing hormone secretion from the adult rat hypothalamus and interaction with cocaine and amphetamine regulated transcript peptide and neuropeptide Y. Regul Pept. **92:** 17–24.
33 Paxinos G, Watson C (1986). The rat brain in stereotaxic coordinates. New York: Academic Press.
34 Rogge G, Jones D, Hubert GW, Lin Y, Kuhar MJ (2008). CART peptides: regulators of body weight, reward and other functions. Nat Rev Neurosci. **9:** 747–758.
35 Sahu A (2003). Leptin signaling in the hypothalamus: emphasis on energy homeostasis and leptin resistance. Front Neuroendocrinol. **24:** 225–253.
36 Smith SM, Vaughan JM, Donaldson CJ, Rivier J, Li C, Chen A et al (2004). Cocaine- and amphetamine-regulated transcript activates the hypothalamic-pituitary-adrenal axis through a corticotropin-releasing factor receptor-dependent mechanism. Endocrinology. **145:** 5202–5209.
37 Stanley SA, Small CJ, Murphy KG, Rayes E, Abbott CR, Seal LJ et al (2001). Actions of cocaine- and amphetamine-regulated transcript (CART) peptide on regulation of appetite and hypothalamo-pituitary axes *in vitro* and *in vivo* in male rats. Brain Res. **893:** 186–194.
38 Thim L, Kristensen P, Nielsen PF, Wulff BS, Clausen JT (1999). Tissue-specific processing of cocaine- and amphetamine-regulated transcript peptides in the rat. Proc Natl Acad Sci USA. **96:** 2722–2727.
39 Tian DR, Li XD, Shi YS, Wan Y, Wang XM, Chang JK et al (2004). Changes of hypothalamic alpha-MSH and CART peptide expression in diet-induced obese rats. Peptides. **25:** 2147–2153.
40 Van Vugt DA, Lujan ME, Froats M, Krzemien A, Couceyro PR, Reid RL (2006). Effect of fasting on cocaine-amphetamine-regulated transcript, neuropeptide Y, and leptin receptor expression in the non-human primate hypothalamus. Neuroendocrinology. **84:** 83–93.
41 Vicentic A, Lakatos A, Jones D (2006). The CART receptors: background and recent advances. Peptides. **27:** 1934–1937.
42 Vrang N, Larsen PJ, Kristensen P, Tang-Christensen M (2000). Central administration of cocaine-amphetamine-regulated transcript activates hypothalamic neuroendocrine neurons in the rat. Endocrinology. **141:** 794–801.
43 Wang ZW, Zhou YT, Kakuma T, Lee Y, Higa M, Kalra SP et al (1999). Comparing the hypothalamic and extrahypothalamic actions of endogenous hyperleptinemia. Proc Natl Acad Sci USA. **96:** 10373–10378.
44 Wierup N, Gunnarsdottir A, Ekblad E, Sundler F (2007). Characterization of CART-containing neurons and cells in the porcine pancreas, gastro-intestinal tract, adrenal and thyroid glands. BMC Neurosci. **11:** 8–51.
45 Yu WH, Kimura M, Walczewska A, Karanath S, McCann SM (1997). Role of leptin in hypothalamic-pituitary function. Proc Natl Acad Sci U S A. **94:** 1023–1028.