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 as 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. 1997). 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 neuroendocrine 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 (2000 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 determined 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.

IV procedure
Intravenous injection of CART resulted in 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 iv 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).

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).

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 | C1 | p-value | CART | C2 | 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
C1 – vehicle (artificial cerebrospinal fluid) injected non-starved rats
C2 - 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 |
| ACTH pg/ml | C1 | p-value | C2 | CART | p-value | CART |
| 271 ± 57 | ns | 228 ± 58 | 270 ± 24 | <0.01 | 195 ± 42 |
| LH ng/ml | 0.52 ± 0.30 | <0.05 | 0.32 ± 0.12 | 0.21 ± 0.04 | <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
C1 – vehicle (artificial cerebrospinal fluid) injected non-starved rats
C2 - 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 \textit{in vitro} experiments, leptin stimulated the release of GnRH from the medial basal hypothalamus, and gonadotropins from the dispersed anterior pituitary cells (Yu \textit{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 \textit{et al.} 2001).

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

\begin{table}[h]
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\hline
 & \textbf{C}_3 & \textbf{Non-starved rats} & \textbf{CART} & \textbf{C}_4 & \textbf{Starved rats} & \textbf{CART} \\
\hline
\textbf{ACTH pg/ml} & & 202 ± 33 & \textit{ns} & 189 ± 71 & 138 ± 16 & \textit{ns} & 131 ± 19 \\
\hline
\textbf{LH ng/ml} & & 0.40 ± 0.31 & \textit{ns} & 0.40 ± 0.30 & 0.17 ± 0.04 & \textit{ns} & 0.22 ± 0.07 \\
\hline
\textbf{FSH ng/ml} & & 6.7 ± 0.75 & \textit{ns} & 6.9 ± 1.2 & 5.9 ± 0.80 & \textit{ns} & 5.8 ± 0.80 \\
\hline
\textbf{PRL ng/ml} & & 5.8 ± 1.8 & <0.05 & 14 ± 11 & 3.0 ± 0.96 & \textit{ns} & 4.4 ± 2.0 \\
\hline
\textbf{GH ng/ml} & & 4.5 ± 1.2 & <0.01 & 25 ± 11.5 & 2.1 ± 1.2 & \textit{ns} & 3.5 ± 2.6 \\
\hline
\textbf{TSH ng/ml} & & 1.0 ± 0.20 & <0.01 & 1.7 ± 0.20 & 0.70 ± 0.10 & \textit{ns} & 0.80 ± 0.20 \\
\hline
\textbf{Corticosterone ng/ml} & & 186 ± 71 & \textit{ns} & 189 ± 63 & 312 ± 130 & \textit{ns} & 210 ± 122 \\
\hline
\textbf{Leptin ng/ml} & & 5.6 ± 1.5 & \textit{ns} & 5.3 ± 1.3 & 0.43 ± 0.11 & \textit{ns} & 0.45 ± 0.12 \\
\hline
\end{tabular}
\caption{Serum pituitary hormones, corticosterone and leptin concentrations at 60 min after iv administration of CART or vehicle in non-starved and starved rats.}
\end{table}

Data are presented as mean ± SD
\( \text{C}_3 \) – vehicle (0.9% NaCl) injected non-starved rats
\( \text{C}_4 \) – vehicle (0.9% NaCl) injected starved rats

\begin{table}[h]
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\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
 & \textbf{C}_3 & \textbf{Non-starved vs. Starved rats} & \textbf{CART} & \textbf{C}_4 & \textbf{Non-starved vs. Starved rats} & \textbf{CART} \\
\hline
\textbf{ACTH pg/ml} & & 202 ± 33 & <0.001 & 138 ± 16 & 189 ± 71 & \textit{ns} & 131 ± 19 \\
\hline
\textbf{LH ng/ml} & & 0.40 ± 0.31 & <0.05 & 0.17 ± 0.04 & 0.40 ± 0.30 & \textit{ns} & 0.22 ± 0.07 \\
\hline
\textbf{FSH ng/ml} & & 6.7 ± 0.75 & \textit{ns} & 5.9 ± 0.80 & 6.9 ± 1.2 & <0.05 & 5.8 ± 0.80 \\
\hline
\textbf{PRL ng/ml} & & 5.8 ± 1.8 & <0.01 & 3.0 ± 0.96 & 14 ± 11 & <0.01 & 4.4 ± 2.0 \\
\hline
\textbf{GH ng/ml} & & 4.5 ± 1.2 & <0.01 & 2.1 ± 1.2 & 25 ± 11.5 & <0.01 & 3.5 ± 2.6 \\
\hline
\textbf{TSH ng/ml} & & 1.0 ± 0.20 & <0.01 & 0.70 ± 0.10 & 1.7 ± 0.20 & <0.001 & 0.80 ± 0.20 \\
\hline
\textbf{Corticosterone ng/ml} & & 186 ± 71 & <0.05 & 312 ± 130 & 189 ± 63 & \textit{ns} & 210 ± 122 \\
\hline
\textbf{Leptin ng/ml} & & 5.6 ± 1.5 & <0.001 & 0.43 ± 0.11 & 5.3 ± 1.3 & <0.001 & 0.45 ± 0.12 \\
\hline
\end{tabular}
\caption{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).}
\end{table}

Data are presented as mean ± SD
\( \text{C}_3 \) – vehicle (0.9% NaCl) injected non-starved rats
\( \text{C}_4 \) – 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 intracerebroventricularly 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-starved 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 hypothalmo-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 hypothalmo-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
8 Broberger C (1999). Hypothalamic cocaine- and amphetamine-regulated transcript (CART) neurons: histochemical relationship to thyrotropin-releasing hormone, melatonin-concentrating hormone, orexin/hypocretin and neuropeptide Y. Brain Res. 848: 101–113
CART and hormone levels in starved rats


