Peptides 91 (2017) 20–25

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

The effect of CART on pituitary hormones release from cultured pituitary cells harvested from fasted and fed ad libitum male rats

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ARTICLE INFO

Article history:
Received 30 October 2016
Received in revised form 23 February 2017
Accepted 7 March 2017
Available online 11 March 2017

Keywords:
CART
Pituitary hormones
Starvation

ABSTRACT

Cocaine and Amphetamine-Regulated Transcript (CART) is widely expressed in the central nervous system and in several endocrine organs. CART is an important factor in the regulation of energy homeostasis. The aim of the study was to assess the role of CART in physiological response of pituitary cells in a course of starvation. The pituitary cells harvested from starved and fed ad libitum male rats were cultured for 48 h and treated with: 0.1 nM, 1 nM, 10 nM or 100 nM doses of CART. The medium was collected after 60 min and stored at −70 °C until samples were further assayed for: LH, FSH, PRL, GH, TSH and ACTH. We revealed that in cultures of pituitary cells collected from fasted rats the basal levels of the examined hormones were reduced. Incubation of pituitary cells of non-starved rats with any dose of CART reduced the concentration of LH and TSH, while the levels of the other hormones were decreased after administration only specific doses of CART. In cells of fasted rats no change in the concentration of gonadotrophins was observed. The PRL level was increased only in the 1 nM dose of CART, while the 10 nM and 100 nM CART doses markedly enhanced GH and TSH. Moreover, administration of 1 nM, 10 nM and 100 nM of CART to cultured cells of fasted rats resulted in a significant rise of the ACTH.

Our results indicate that CART can directly affect the physiological release of PRL, ACTH, TSH and GH in pituitary cells of starved animals. Moreover, CART did not alter the LH and FSH suppression level, which is correlated with food deprivation. This data stays in contrast with the already proposed role of CART as an anorexigenic hypothalamic factor.

1. Introduction

In 1995, a previously unknown mRNA transcript was identified in the rat striatum. Interestingly, its expression level increased five-fold after cocaine and amphetamine injections. This transcript was named Cocaine and Amphetamine-Regulated Transcript and the protein encoded by it was called CART [18]. Biologically active peptide fragments, CART55-102 and CART62-102 in rodents, as well as CART42-89 and CART49-89 in humans are the products of tissue-specific posttranslational proCART modification [27]. The CART mRNA sequence is highly conserved across different species. In humans, CART is widely expressed in the cells of the central nervous system and in several endocrine organs. Immunohistochemical studies revealed the presence of CART in the hypothalamus, pituitary and adrenal glands as well as in the pituitary portal circulation. CART expression was mainly observed in gonadotrophs and lactotrophs and in corticotrophs, thyrotrophs and somatotrophs [15,22,28,43,46].

The in vivo data indicates that CART plays a significant role in the modulation of hypothalamic peptides secretion, such as: gonadotropin-releasing hormone (GnRH), growth hormone-releasing hormone (GHRH), corticotrophin releasing hormone (CRH) and thyrotropin releasing hormone (TRH). It has also been reported that CART stimulates secretion of CRH, TRH and neuropeptide Y (NPY) from hypothalamic explants [27]. Additionally, Broberger et al. showed co-existence of CART with other neuropeptides, such as: TRH, melanin-concentrating hormone (MCH), and NPY in the hypothalamus [8].

Moreover, CART expression in pituitary cells enables its paracrine action that modifies the synthesis and secretion of trophic hormones. Our previous results and other studies showed that CART may influence pituitary hormones release [5,47]. Shortage of food induces adaptive changes in the hypothalamo-pituitary axis leading to lower energy expenditure. Cumulative

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http://dx.doi.org/10.1016/j.peptides.2017.03.003
0196-9781/

data indicates that starvation in both animals and humans activates the hypothalamic–pituitary–adrenal axis and inhibits the hypothalamic–pituitary–gonadal axis. These changes may partially be due to the complex alterations of orexigenic and/or anorexigenic peptides activity [2,4,11,12,32,34]. Generally, during fasting the activity of peptides involved in the control of energy homeostasis and in the mechanism of hormones release is changed, and CART secretion in the hypothalamic nuclei is reduced [2,6,26,51]. It was proposed that during energy deprivation the response of pituitary cells to neuropeptides action might also be altered.

Both the CART mRNA and peptide have been detected in the structures directly involved in food intake and neuroendocrine regulation and it is generally accepted that CART presents anorexigenic potency [21,48]. Some of the published in vivo studies demonstrated that CART regulates energy homeostasis and influences the mechanism of central and peripheral neuroendocrine control of appetite through interaction with other peptides at the hypothalamic level [14,28,31,38].

Our previously published in vivo data suggested that CART modulated pituitary hormonal profile in non-starved rats [1]. However, the effect of CART was dependent on administration method, as differences in the results were observed when CART was given centrally (icv) or peripherally (iv). In contrast, in a course of food limitation CART did not change the levels of pituitary hormones [13].

Overall, to better understand the role of CART in the pituitary cells secretion during energy deprivation, we performed studies using the in vitro tissue culture model. The aim of the study was to assess whether CART can directly affect the physiological response of pituitary cells in a course of starvation. The obtained data provides new insight into the mechanisms of pituitary hormone secretions during fasting.

2. Materials and methods

2.1. Animals

Male Wistar rats (240–260 g) were maintained under controlled conditions: 14:10 light–dark cycle (lights on at 06:00 AM); controlled temperature (23 ± 1 °C), with free access to laboratory chow/food and tap water. Animals were housed three per cage and after one week of acclimatization they were divided into two experimental groups. One group of rats ($n = 50$) was strictly fasted for 72 h with free access to water only and the other group ($n = 50$) was fed ad libitum. The starvation lasting for 72 h and its effect on hormonal regulation was investigated by other authors [23,55].

All the procedures were approved by the IV Animal Use and Care Ethics Committee, National Institute of Public Health, Warsaw, Poland.

2.2. Dispersion of pituitary

The procedures of pituitary tissue dissociation, cell preparation and cell culture were performed as previously described [4,45]. Briefly, rats were anesthetized with an intraperitoneal injection of ketamine in a dose of 300 mg/kg and sacrificed. Pituitary glands were quickly dissected and anterior lobes of the pituitaries (APs) were washed twice with Dulbecco's modified Eagle's medium (DMEM), supplemented with 0.3% fetal calf serum (FCS), 0.01% antibiotic/antimycotic (AA), and then immediately processed for culturing in medium (DMEM, 5% FCS, AA). AP cells were dispersed with 0.1% trypsin in DMEM for 15 min at 37 °C and incubated in DMEM supplemented with 0.1% DNase I (deoxyribonuclease I from bovine pancreas, type IV), 10% FCS and 0.01% antibiotic/antimycotic for next 15 min. Then, cells were mechanically dispersed through a sieve (300 μm/50 meshes), washed twice with culture medium and centrifuged for 15 min at 2000 rpm. Thereafter, AP cells were resuspended in culture medium, counted and assessed for viability by trypan blue exclusion. Percentage of viable cells was >97. Finally, cells were seeded (5×10^5 cells/well) into 24-well format culture plates and maintained for 48 h at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. To examine the effects of CART, the seeded cells were cultured for 60 min in: DMEM supplemented with: 0.1 nM, 1 nM, 10 nM, 100 nM doses of CART (55-102) and in pure medium (no CART added; control group). CART doses were selected based on the previously published data [13,16,40].

At the end of experiment, media samples were collected and stored at −70 °C until further analysis for luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), growth hormone (GH), thyroid-stimulating hormone (TSH), and adrenocorticotropic hormone (ACTH) was performed.

Table 1
Basal pituitary hormones concentration (mean ± SD) in cultured pituitary cells harvested from fed ad libitum or fasted rats.

Hormone	Fed ($N = 10$)	Fasted ($N = 10$)	p value
LH, ng/ml	39.4 ± 3.9	13.7 ± 1.9	<0.001
FSH, ng/ml	14.0 ± 1.3	7.5 ± 4.2	<0.01
PRL, ng/ml	105 ± 41.8	48.4 ± 12.7	<0.01
GH, ng/ml	753 ± 132.6	475 ± 66.6	<0.001
TSH, ng/ml	10.2 ± 1.6	3.1 ± 0.9	<0.001
ACTH, pg/ml	3553 ± 356	1234 ± 73.4	<0.001

2.3. Hormone analyses

Concentrations of LH, FSH, PRL and TSH in cell culture medium were measured by radioimmunoassay (RIA) using reagents prepared by A.F. Parlow and provided by the NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA). Values were expressed in relation to NIDDK-rLH-RP-3, NIDDK-rFSH-RP-2, NIDDK-rPRL-RP-3 and NIDDK-rTSH-RP-3 reference standards. The limit of detection varied for individual hormones and was set up at: LH, 0.1 ng/ml; FSH, 1.25 ng/ml; PRL, 0.39 ng/ml and TSH, 0.3 ng/ml. Concentrations of GH were measured with the RIA method using Linco Research kit, (St. Charles, MO, USA). The sensitivity of the assays was 0.5 ng/ml. Concentrations of ACTH were determined with RIA method using a commercial kit from Phoenix Pharmaceuticals USA (Burlingame, CA, USA). The detection limit of the assay was 10 pg/ml. All samples were analyzed in a single assay. In all analyses the intra-assay coefficients of variation (CV) were below 10%.

2.4. Statistical analysis

All results were analyzed using Statistica 10 (StatSoft, Inc., USA) software and data is expressed as mean and standard deviation (±SD). Statistical evaluation of data was performed using the Kruskal–Wallis rank test or Mann–Whitney U test. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Analysis of the basal level of hormones secreted by pituitary cells harvested from starved or fed ad libitum male rats

To establish the basal level of hormones, pituitary cells were harvested from both: fasted and fed rats, then cultured and analyzed. The obtained data (Table 1) showed that rats fasted for 72 h demonstrated strong reduction (up to 3-fold) in basal level of secreted hormones. The release of TSH, ACTH and LH was reduced by ~70%,