

Direct effects of cocaine-amphetamine-regulated transcript (CART) on pituitary hormone release in pituitary cell culture

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Abstract

OBJECTIVE: Cocaine- and amphetamine-regulated transcript (CART) is widely expressed in the rat brain, especially in the hypothalamic nuclei and in the anterior pituitary. The aim of this study was to evaluate the effects of CART on pituitary hormone release in pituitary cell culture.

MATERIAL AND METHODS: The pituitary hormone release from pituitary cell culture after CART administration was investigated. Concentrations of LH, FSH, PRL, TSH and GH were measured with RIA methods.

RESULTS: CART in all doses (1nMol, 10nMol, 100nMol) stimulated prolactin (PRL) release and inhibited TSH release. CART administration caused a dose dependent decrease in LH release. CART did not change GH release from cultured pituitary cells.

CONCLUSION: CART may affect directly pituitary hormones release in the cell culture.

Introduction

Cocaine- and amphetamine-regulated transcript (CART) is widely expressed in hypothalamic nuclei [1, 2, 3, 4]. CART mRNA is also expressed in the brain stem and spinal cord [1, 5], the anterior pituitary [5] the adrenal gland [5], and D cells in the pancreatic islet of Langerhans [6].

It has been known that CART plays a physiological role in feeding behaviour. Intracerebroventricular (icv) administration of recombinant CART inhibits food intake in the rat [7, 8]. The metabolic state may affect CART mRNA; fasting reduces the expression of CART in the arcuate nucleus (Arc

[7]. CART mRNA is colocalized with vasopressin and corticotrophin-releasing factor containing neurons [9]. The effect of CART on CRH neurons leads to corticosterone secretion from the adrenal gland [10]. Intracerebroventricular (icv) injection of CART activated neurons in the paraventricular nucleus (PVN), rich in corticotrophin-releasing factor (CRH) and thyrotrophin-releasing factor (TRH) [11]. These results may suggest that CART may play a role in the neuroendocrine functions. The aim of this study was to evaluate direct effects of CART on pituitary hormones release from cultured pituitary cells.

Material and Methods

In the study we used adult Wistar-Kyoto male rats (3-months old). The animals were maintained under standard laboratory conditions on 12/12 h light/dark cycle (lights on at 07.00 h). Food and water were available *ad libitum*. All experiments were approved by Local Ethical Committee for The Care and Use of Experimental Animals.

The procedure of pituitary tissue dissociation, cell preparation and cell culture were based on methods described previously [12,13,14]. Briefly, pituitary glands were obtained from three-month-old (weight app. 200 g) male WKY rats, anesthetized by pentobarbitalum vetbutal injection and decapitated. They were washed twice with DMEM pH 7.3 with 0.2% glucose, 2 mmol glutamine/l, 0.3% bovine serum albumin (BSA), penicillin (50 U/ml) and streptomycin (50 µg/ml) and processed for culture immediately. They were enzymatically dispersed during 20 min incubation at 37°C in 0.1% trypsin in PBS buffer (without Ca²⁺ and Mg²⁺) followed by 20 mins of incubation in 0.1% DNA-se I (deoxyribonuclease I from bovine pancreas, type IV) in DMEM pH 7.3 with, 0.3% BSA, penicillin (50 U/ml) and streptomycin (50 µg/ml). The glands were finally mechanically dispersed on a sieve (50 mesh) and washed twice by centrifugation for 10 min at 50 g with culture medium (DMEM pH 7.3 with 0.2% glucose, 2 mmol glutamine/l, 0.3% BSA and 10% fetal calf serum (FCS)). The pituitary cells were counted in a hemocytometer and assessed for viability by exclusion of trypan blue (>85%).

The pituitary cells (0.2 x 10⁶/ml) were incubated in 24-well culture plates for up to 48 hrs in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. The culture plates were washed with twice the volume of the serum-free medium with 30 µg ascorbic acid/l 30 mins before every experiment. The neuropeptides were dissolved in saline at concentrations 1 mmol/l. They were diluted with serum-free medium with 30 µg ascorbic acid /l to final nanomolar concentrations.

For short-term effects, CART in doses 1 nMol, 10 nMol, 100 nMol were added after 48 hrs of culture and the medium was collected 30, 60, 120, 240 and 480 mins thereafter. The collected medium was stored at -20°C until assayed for LH, FSH, PRL, GH and TSH.

All media and chemicals were purchased from Sigma (Sigma Aldrich Chemie GmbH, Deisenhofen, Germany) and culture dishes from Corning (Bibby Sterlin Ltd, Staffordshire, UK). The experiments were repeated 3 times, 2 x 10⁵/ml cells were present in each culture.

Hormone measurements

For each hormone all plasma samples were measured in the same RIA procedure. Concentrations of LH, FSH and PRL were measured by RIA using reagents prepared by Dr. A.F. Parlow and provided by the NIDDK (Bethesda, MD). Values were expressed in terms of the LH-RP3, FSH-RP2 and PRL-RP3 of the reference standard, respectively. The detection limit for LH, FSH and PRL assay was 0.1 ng/ml, 0.6 ng/ml

and 0.5 ng/ml, respectively. Plasma concentrations of the rat TSH and GH were measured by kits provided by Biocode S.A., France. The limit of detection for TSH was 0.1 ng/ml and 1 ng/ml for GH ng/ml. In all kits the intra-assay coefficients of variation were less than 9%.

Results

Effects of CART on pituitary hormones release (rLH, rFSH, rPRL, rTSH, rGH) from cultured pituitary cells were demonstrated in *Table 1*. CART administration caused a dose dependent decrease in LH release after 120 min incubation. CART in all doses (1 nM, 10 nM, 100 nM) stimulated PRL release after 120 mins and in doses 10 nM, 100 nM after 240 min incubation. CART in all doses (1 nM, 10 nM, 100 nM) inhibited TSH release after short time of incubation (60 mins). However, CART did not change GH release from cultured pituitary cells.

Discussion

The role of CART in the mechanism of pituitary hormones secretion has not been understood yet. Our previous studies showed that intracerebroventricular injection of CART leads to an increase of prolactin (PRL), and GH release, however, intravenous (i.v) administration of CART stimulated PRL, GH and TSH release [18].

The present studies revealed the direct effects of CART on pituitary hormones release in cell culture. CART in all doses stimulated PRL release after 120 min and 240 min incubation. CART inhibited in a dose dependent manner TSH release after short incubation (60 min). However, CART did not change GH release from cultured pituitary cells. CART inhibited LH release in a dose dependent manner after 120 min incubation. Previous data indicated that intracerebroventricular (icv) injection of CART increases LH release [18].

Our results demonstrated that CART administered centrally (icv) and peripherally (i.v) markedly increases PRL release. The stimulation of PRL release was also found after the direct action of CART in the cell culture. The opposite effects of CART were observed in LH, TSH release during experiments "*in vivo*" and "*in vitro*". An increase in GH release was found after icv and i.v injections of CART however, in the cell culture CART did not affect GH. The effects of CART on pituitary hormone release are controversial. The icv injection of CART in a dose which significantly reduces food intake inhibits PRL and GH [11]. Hypothalamic CART neurons coexpress neuropeptides involved in energy homeostasis including MCH, TRH, Dyn and NT [4]. In the hypothalamic nuclei CART is colocalized with vasopressin and CRH containing neurons [9]. Moreover, CART stimulated CRH, TRH and NPY from hypothalamic experiments *in vitro* [11].

It has been known that leptin, NPY and other neuropeptides affects feeding metabolism and neuroendocrine regulation [15, 16, 17]. Our previous results suggest that effects of CART on pituitary hormones

Table 1. EFFECTS OF CART ON PITUITARY HORMONES RELEASE IN PITUITARY CELL CULTURE

	rLH ng/ml	rFSH ng/ml	rPRL ng/ml	rTSH ng/ml	rGH ng/ml
AFTER 60 mins.					
CONTROL	104.7 ± 8.6	74.9 ± 3.0	534 ± 30	85.9 ± 11	2380 ± 174
CART 1nM	92.6 ± 18.3 (p<0.05)	80.4 ± 3.0	592 ± 40	65.8 ± 9.0 (p< 0.05)	2301 ± 124
CART 10nM	97.5 ± 11.1	75.6 ± 3.3	446 ± 28	58.2 ± 3.3 (p< 0.01)	2276 ± 74
CART 100nM	136.3 ± 8.6	74.9 ± 3.1	431 ± 43	47.0 ± 3.3 (p<0.01)	2354 ± 109
AFTER 120 mins.					
COTROL	131.3 ± 7.0	88.9 ± 6.0	354 ± 30	57.6 ± 5.0	2355 ± 166
CART 1nM	120.0 ± 3.1 (p<0.05)	69.8 ± 3.0	448.6 ± 50 (p< 0.05)	66.6 ± 5.7	2113 ± 144
CART 10nM	96.1 ± 4.0 (p<0.01)	83.0 ± 3.0	593 ± 27 (p<0.01)	81.1 ± 8.0	2456 ± 241
CART 100nM	86.6 ± 9.9 (p<0.01)	80.4 ± 2.0	533.4 ± 28 (p< 0.01)	71.4 ± 8.0	2534 ± 135
AFTER 240 mins.					
CONTROL	101.7 ± 1.9	69.5 ± 3.0	295 ± 5.2	64.8 ± 8.0	2441 ± 180
CART 1nM	95.3 ± 20.7	68.8 ± 2.5	343 ± 9.9	60.4 ± 4.5	2376 ± 134
CART 10nM	99.9 ± 6.2	63.9 ± 2.0	406 ± 12.3 (p<0.01)	56.2 ± 4.0	2415 ± 94
CART 100nM	106.8 ± 4.0	71.6 ± 2.8	394 ± 7.5 (p<0.01)	58.9 ± 7.5	2319 ± 53

release may be mediated by leptin [18]. The modulation of CART action by other neuropeptides may explain the different effects of CART on pituitary hormones release in experiments *in vivo* and *in vitro*.

Conclusion

CART may affect directly pituitary hormones release in the cell culture.

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