Controversial opinions on the role of cocaine and amphetamine-regulated transcript (CART) in prolactin release

Boguslawa Baranowska, Agnieszka Baranowska-Bik, Wojciech Bik, Ewa Wolinska-Witort, Lidia Martynska & Magdalena Chmielowska

Neuroendocrinology Department, Medical Centre of Postgraduate Education, Warsaw, Poland

Correspondence to: Prof. Boguslawa Baranowska MD., PhD.
Neuroendocrinology Department, Medical Centre of Postgraduate Education
Marymoncka 99/103, 01-813 Warsaw, Poland
PHONE: +48 22 56 93 850
FAX: +48 22 56 93 859
EMAIL: zncmkp@op.pl; zne@cmkp.edu.pl

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Summary

Cocaine and amphetamine regulated transcript (CART) is widely expressed in the central nervous system and in several endocrine organs. The physiological role of this peptide includes modulation of appetite control, energy expenditure, thermoregulation and hormone secretion. It has been suggested that CART influences prolactin (PRL) secretion both directly and indirectly. However, the mechanism underlying the regulation of PRL release by CART remains unclear.

REVIEW

Cocaine and amphetamine regulated transcript (CART) is widely expressed in the central nervous system as well as in several endocrine organs but is particularly concentrated in the hypothalamus [1]. It is co-localized with known neurotransmitters and neuropeptides that are very important in the control of appetite, energy expenditure, thermoregulation and in the mechanism of hormone secretion [2,3,4]. CART peptides, particularly CART (55-102), appear to participate in a regulation of energy homeostasis. It has been found that intracerebroventricular (icv) administration of CART (55-102) reduces appetite and stimulates energy expenditure [5]. Moreover, CART mRNA and the presence of CART immunoreactivity has been reported in hypothalamic nuclei that are known to modulate pituitary function [1]. Additionally, CART peptides have also been localized in the anterior pituitary gland itself but its role in pituitary function has not yet been elucidated [6]. Furthermore, CART enhances the release of corticotrophin-releasing hormone (CRH), thyrotrophin-releasing hormone (TRH) and neuropeptide Y from the hypothalamic explants and reduced αMSH release [7]. It has also been reported that CART stimulates GnRH pulse secretion from prepubertal female rat hypothalamic tissue *in vitro* [8,9]. The central administration of CART activates CRH neurons in the rat paraventricular nucleus (PVN) and results in a rise of ACTH and corticosterone [7,10,11]. These results suggest that CART peptide may activate hypothalamic-pituitary-adrenal, -thyroid and -gonadal axes.

CART is one of the many peptides synthesized in the arcuate nucleus where it plays an important role in energy homeostasis. It is also expressed in the paraventricular nucleus. Besides, its presence has

been demonstrated in neuroendocrine and sympathetic neurons [12,13]. CART neurons are concentrated in the anterior periventricular zone where are co-expressed with somatostatin [12,14,15], and in the medial parvocellular subdivision of the PVN with CART neurons being co-localized with TRH [3,13]. Moreover, the parvocellular division of the PVN is an area rich in neurons containing peptides important in the neuroendocrine regulation: CRH, TRH, vasoactive intestinal peptide (VIP) and galanin [16,17]. In addition to the coexistence of CART and TRH in hypophysiotropic neurons, CART is also expressed in other cell types that might influence lactotrops [2,18,19].

It has been suggested that CART may exert its effects on anterior pituitary hormones production not only by secretion into the portal system but also through paracrine and/or autocrine regulation [19]. Prolactin (PRL) has been known to be tonically inhibited by dopamine released from the tuberoinfundibular dopaminergic (TIDA) neurons [20]. According to the previous studies, it could be stated that CART peptide may modulate the dopamine (DA) system. However, opinions on the effects of CART on PRL release remain equivocal. Some authors have reported that CART may stimulate prolactin centrally via inhibition of dopaminergic neurons or even through an increase in action of PRL releasing peptides [20,21]. Stanley *et al.* demonstrated that icv injection of CART increases serum PRL levels in male rats [7]. These authors suggested that this stimulatory effect is due to an increase in hypothalamic TRH, which is also a prolactin releasing peptide [7,22]. Moreover, injection of CART was also found to enhance TRH release from medial hypothalamic explants [7].

Another mechanism of PRL stimulation is linked with vasoactive intestinal peptide (VIP). Besides, the co-localization of CART and VIP has been well established. Kato *et al.* [23] were the first to report that PRL release is stimulated by VIP, both *in vivo* and *in vitro*. Currently, VIP is considered to be a PRL releasing factor in birds, animals and humans [24,25,26,27].

In addition, some studies have shown that CART inhibits hypothalamic dopamine release from synaptosomes [28]. In contrast to these findings, other authors demonstrated that central injection of CART increases DA turnover in some hypothalamic DA regions in vivo [29]. Kuriyama *et al.* [6] showed that CART peptide suppresses prolactin release from dispersed anterior pituitary cells.

CART innervates the hypophysiotropic TRH neurons and, as well, is co-released with TRH into the pituitary portal circulation. Fekete *et al.* demonstrated that CART inhibits the stimulatory effect of TRH on prolactin release but has no influence on TRH-induced increase of TSH release and CART itself does not modulate prolactin production in pituitary cells [30]. Furthermore, Raptis *et al.* indicated that CART *in vitro* has an important role in the modulation of TRH-induced prolactin secretion. These authors also concluded, analyzing the results of in situ hybridization, that enhanced synthesis of this peptide may take part in the mechanism of reduced TRH-induced prolactin response during hypothyroidism [31]. Besides, CART is able to inhibit TRH-induced PRL release but not TRH-induced TSH release in adenohypophyseal cell cultures.

According to the co-localization of TRH and CART neurons, some authors conducted studies on the influence of neural stimuli, like suckling or cold exposure, on TRH and CART mRNA expression levels and release of PRL and TSH. It has been demonstrated *in vivo* that both suckling and cold exposure increase TRH mRNA in the PVN, but only suckling induces PRL secretion [32]. Moreover, it has been found that CART mRNA level is enhanced only after cold exposure. Thus, the studies of Sanchez *et al.* [32] demonstrated the differential regulation of CART gene expression in hypophysiotropic neurons in response to stimuli that increase TRH mRNA. These authors also suggested that CART activation in the PVN may lead to the decrease in PRL and increase in TSH secretion in low temperature conditions.

It has also been found that there is a close link between CART mRNA expression and lactation. Recent studies of Smith *et al.* [33] revealed that in lactating rats the concentration of CART mRNA in the anterior pituitary and supraoptic nucleus is increased. Moreover, the circulating levels of CART peptide are also elevated as compared with non-lactating rats. In addition, a significant inhibition of CART gene expression and peptide release is observed after treatment with bromocriptine in pituitary cell culture [33]. Therefore, it could be suggested that CART is a important factor in the regulation of lactation.

Apart from its central action, CART is also able directly to stimulate pituitary cells [7]. Our previous studies demonstrated the stimulation of PRL release in response to CART administration *in vivo* and *in vitro* in male rats [34,35]. However, in literature data concerning the effect of CART peptide on hypothalamic DA systems remain unclear. Initially, some researchers have shown that the turnover of tuberoinfundibular dopaminergic (TIDA) neurons exhibits diurnal changes in female but not in male rats, and these activity changes may be related to the control of cholinergic system [36]. Nevertheless, very important but unanswered question is whether the effects of CART on hypothalamic TIDA are gender dependent. The results of the studies by Yang *et al.* [37] indicated that stimulation by CART on TIDA neurons is not influenced by gender. On the other hand, it is well recognized that estrogens, similarly to thyroid hormones, may modulate the activity of prolactin releasing peptide. These controversial findings may be partly explained by the co-existence and co-expression in PVN and other nuclei of CART and many more neuropeptides that are involved in the regulation of hormonal secretion by direct action on pituitary cells or by an indirect effect on TIDA activation.

It has previously been shown that CART is present in the parvocellular division of the PVN, an area rich in

neurons containing peptides important in the control of neuroendocrine regulation and thermoregulation such as CRH, TRH, VIP, galanin, NPY, α-MSH and dynorphin [16,17,38,39,40]. It has also been demonstrated that CART markedly stimulates the release of CRH, TRH and NPY from hypothalamic explants *in vitro* [7].

CART peptide may be able to regulate PRL secretion in multiple ways and therefore this very interesting but controversial subject requires further intensive investigations.

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