Cocaine- and amphetamine-regulated transcript: a possible regulator of the stress response

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Abstract

Introduction

Cocaine- and amphetamine-regulated transcript peptides have been implicated in the endocrine and autonomic regulation, food intake and anxiety. Anatomical studies demonstrate that cocaine- and amphetamine-regulated transcript mRNA and peptides are expressed intensely in the hypothalamo-pituitary-adrenal gland axis and sympatho-adrenal system. In parallel with the anatomical studies, functional studies show that cocaine- and amphetamine-regulated transcript peptides increase the activity of the hypothalamo-pituitary-adrenal axis and sympatho-adrenal system. Also, various stress procedures regulate cocaine- and amphetamine-regulated transcript peptide and mRNA expression in the frontal cortex, amygdala, hypothalamus and adrenal gland. Based on these data, this review discusses the possible role of cocaine- and amphetamine-regulated transcript peptides in the regulation of the stress response.

Conclusion

There is substantial literature which shows that cocaine- and amphetamine-regulated transcript has significant effects on the hypothalamo-pituitary-adrenal axis and sympatho-adrenal system activity. Although increasing number of studies report that cocaine- and amphetamine-regulated transcript expression is regulated by the stress response, the exact role of cocaine- and amphetamine-regulated transcript during the stress response appears to be complex and remains unclear.

Introduction

In 1995, Douglass et al.\textsuperscript{1} demonstrated that an unknown mRNA was up-regulated in the rat striatum by acute administration of psychostimulant drugs, cocaine and amphetamine. This transcript was defined as cocaine and amphetamine regulated transcript (CART). Studies by Douglass et al.\textsuperscript{1} have also characterised the rat and human CART gene. The research showed that transcription of the CART gene yields two alternatively spliced mRNAs resulting in the production of two pro-peptides, proCART 1–89 and proCART 1–102. Neurons in the rat brain express both of these pro-peptides, whereas neurons in the human brain express only the short form. Pro-peptide sequences are further processed to yield smaller and biologically active CART peptide fragments such as CART 55–102, derived from the long form (also named as CART 42–89, derived from the short form) and CART 62–102, derived from the long form (also named as CART 49–89, derived from the short form).

Since 1995, considerable amount of data has accumulated on the anatomical localisation and the functional roles of CART peptides in the brain. CART mRNA and peptides are expressed intensely in the neurons located in the piriform cortex, ventral striatum, amygdaloid complex, bed nucleus of stria terminalis, hippocampal formation, hypothalamus, brain stem (A5, A7, C1, locus ceruleus) and the spinal cord\textsuperscript{3,4}. In addition to the central nervous system, CART peptides are also demonstrated in the sympathetic preganglionic neurons as well as in the endocrine glands such as pituitary, adrenal medulla and pancreas\textsuperscript{5,6}. To date, CART peptides have been implicated in reward and reinforcement, endocrine and autonomic regulation, food intake and anxiety\textsuperscript{7–10}. Additionally, a recent study by Mao et al.\textsuperscript{11} suggests that CART peptides may have strong therapeutic properties during oxidative stress. The aim of this review was to discuss cocaine-and amphetamine-regulated transcript as a regulator of the stress response.

Discussion

The author has referenced some of his own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies. Animal care was also in accordance with the institution guidelines.

Distribution, expression and related functions of cart

CART in the hypothalamus

Hypothalamus integrates endocrine and autonomic functions associated with homeostasis. It has a critical role in the adaptation of the organism to homeostatic challenge. Particularly, paraventricular (PVN) and arcuate (ARC) nuclei, lateral hypothalamic (LHA) and dorsomedial (DMH) areas have attracted considerable attention...
and are implicated in the regulation of the HPA axis, autonomic nervous system activity and energy homeostasis. Corticotrophin releasing hormone (CRH) synthesizing neurons of PVN play a central role in the regulation of the stress response. These neurons are richly innervated by specific cell populations from the ARC, DMH and LHA.

In the central nervous system, CART mRNA and peptides are expressed most intensely in the hypothalamus. Highest densities of CART immunoreactive (IR) cells are present in the PVN, supraoptic nucleus (SON) and ARC. DMH and LHA contain relatively lower density of CART-IR cells.

In the PVN, the medial parvicellular subcompartment (mpPVN) contains neuroendocrine cells which synthesise CRH and project to the external layer of median eminence (ELME). Alternatively, dorsal parvicellular PVN (dpPVN) and the ventral part of medial parvicellular (vmpPVN) subcompartments are associated with autonomic functions. dpPVN harbours presympathetic neurons projecting to the preganglionic cells of the spinal cord, whereas vmpPVN harbours neurons projecting to the dorsal motor nucleus of the vagus. CART-IR neurons reside in all parvicellular subcompartments. In the mpPVN, CART co-localises extensively with thyrotropin-releasing hormone (TSH) and corticotropin (CORT) levels in blood. Additionally, ICV CART induced phosphorylation of cAMP response element binding protein in the CRH neurons of the mpPVN, suggesting the regulation of CRH at the transcriptional level. Furthermore, a study by Mao and Jacks reported a direct transcriptional activity for CART.

A study by Vrang et al. showed that dpPVN contains high amount of CART-IR fibres and they formplexus which surround the parvicellular oxytocin-IR neurons. These presym pathetic neurons express c-Fos after ICV CART injections. Matsumura et al. showed that ICV CART injections elevated arterial blood pressure, plasma epinephrine and norepinephrine levels and renal sympathetic nerve activity. These studies suggest that CART signalling through dpPVN may trigger presynaptic neuron activity.

ARC, LHA and DMH may relay information associated with energy balance to PVN neurons. They also regulate the HPA axis and autonomic nervous system activity. In the ARC, CART/POMC neurons and neuropeptide Y (NPY)/Agouti-related peptide (AgRP) neurons comprise of two distinct populations. Some of the axons projecting to PVN originate in the CART-IR ARC neurons. Some of the CART-IR axon terminals, which end in close proximity to the PVN CRH neurons, contain α-melanocyte-stimulating hormone (α-MSH, peptide product of POMC gene). These results imply that POMC/CART neurons in the ARC may project to the PVN. CART/α-MSH-IR axons are mainly concentrated in the dpPVN, whereas the density of the fibres is less frequent in the medial parvocellular PVN. α-MSH and CART have similar effects on the HPA axis and sympathetic activity. Intra-PVN α-MSH or CART injections, both, increased plasma ACTH and CORT levels. In rodents, CART is absent in α-MSH-synthesizing neurons, but expressed in NPY/AgRP neurons in the human infundibular nucleus (ARC in rodents).

In the LHA and DMH, melanin concentrating hormone (MCH) and orexin neurons constitute two distinct populations. CART neurons in both nuclei extensively co-store MCH and GABA whereas there is no co-localisation with orexin. Some of the CART neurons co-expressing MCH in LHA and DMH innervate PVN. Similar to CART, intraPVN MCH administration is reported to trigger the HPA axis activity causing a significant rise in the plasma ACTH and CORT. Interestingly, although CART and GABA are co-expressed in some LHA and DMH neurons, CART and GABA are suggested to exert divergent effects on the HPA axis. Studies report that axonal projections from the GABAergic neurons of DMH inhibit parvicellular PVN neurons.

Leptin increases thermogenesis and energy expenditure as a result of increased sympathetic activity. Studies suggest that one of the
downstream targets of leptin is hypothalamic CART\textsuperscript{20,30}. Leptin activates CART neurons in PVN, ARC and DMH. Further, leptin activated-CART neurons in ARC project to the SPN of the spinal cord.

**CART in the pituitary**

In the anterior pituitary, lactotrophic, gonadotrophic and corticotrophic cells exhibit moderate CART-IR\textsuperscript{21–23}. Additionally, a high density of CART-IR axonal fibres is present in the posterior pituitary\textsuperscript{5}. These axonal endings may originate from the magnocellular division of PVN and SON. Functional studies show that CRH increases CART secretion from the anterior pituitary segments and CART peptides are found in systemic circulation\textsuperscript{73}.

Interestingly, CART, when administered peripherally, does not increase blood ACTH in contrast to ICV administrations\textsuperscript{34}. Based on this result, the authors suggest that CART activates the HPA axis through the hypothalamus and a CRF-dependent central mechanism.

**CART in the sympatho-adrenal system**

Sympatho-adrenal system is another key component activated during the integrated stress response. Presympathetic neurons in various hypothalamic (PVN, ARC, LHA, ret-rocachiasmatic area (RCA)) and brain stem (parabrachial nucleus, locus ceruleus, A5, rostral ventrolateral medulla (RVLM, C1), medullary raphe, NTS) nuclei regulate the activity of the sympatho-adrenal system\textsuperscript{20,35}. Varying densities of CART-IR neurons and axons are found in these hypothalamic areas and brainstem regions\textsuperscript{4,5,14}.

Hypothalamic nuclei regulating the autonomic activity project either directly to the sympatho-adrenal preganglionic neurons (SNP) or to the brainstem catecholaminergic neurons which innervate SNP. Retrograde tracing studies showed that CART-IR neurons in the ARC, RCA and RVLM (C1) project directly to the intermediolateral cell column (IML) in the spinal cord\textsuperscript{8,30}. Studies show that descending efferent pathways of PVN, ARC, LHA and DMH that terminate in RVLM, NTS, dorsal vagal complex, nucleus ambiguus, raphe nuclei and parabrachial nucleus, are key autonomic centres in the brainstem\textsuperscript{20,21,36–38}. All of these regions contain high/moderate densities of CART-IR cells and axonal fibres\textsuperscript{4,5}. In the RVLM, locus ceruleus and A5 area, most of the catecholaminergic neurons co-express CART\textsuperscript{39,40}. Yoon and Lee\textsuperscript{41} showed that some of the CART neurons which co-contain MCH in the LHA and DMH project to the monoaminergic neurons in the dorsal raphe and locus coeruleus. Functional studies indicate that CART signalling in the hypothalamus and brainstem may regulate the activity in the sympatho-adrenal system. Nitroprusside-induced hypotension increased Fos-IR in barosensitive catecholaminergic CART neurons in the RVLM\textsuperscript{39}. ICV CART increased c-Fos expression in the parabrachial nucleus and NTS\textsuperscript{42}. Intracisternal CART activated RVLM neurons\textsuperscript{43}. Further, ICV\textsuperscript{39} and intracisternal\textsuperscript{41} CART injections increased heart rate, blood pressure, renal sympathetic nerve activity and plasma adrenaline levels whereas intrathecal CART administration enhanced the pressor response induced by glutamate\textsuperscript{44}.

In the spinal cord, SPN which reside in the IML are CART-IR\textsuperscript{5}. Almost all of these CART neurons are also cholinergic. CART is not expressed in the parasympathetic preganglionic neurons. CART (+) axonal endings of the SPN terminate on the postganglionic neurons in the paravertebral and prevertebral ganglia. However, these postganglionic neurons do not express CART. In the superior cervical and stellate ganglia, CART-IR axon terminals of the CART synthesizing postganglionic neurons innervate the vasoconstrictor and heart-projecting postganglionic cells\textsuperscript{45}. Additionally, CART labelling is also demonstrated in the SPN projecting to the celiac ganglia known to innervate digestive tract structures including liver and pancreas\textsuperscript{46,47}. Parker et al\textsuperscript{47} reported that glucoprivation induced c-Fos labelling in many CART mRNA (+) SPN in the C8-T3 level projecting to the superior cervical ganglion. This may contribute to the increased heart rate and cardiac output observed during acute hypoglycaemia.

**CART in the adrenal gland**

In the adrenal gland, CART-IR axons of the SPN form a plexus under the adrenal capsule and terminate in the medulla surrounding only the noradrenergic chromaffin cells\textsuperscript{45,46}. CART peptides are expressed only in the noradrenergic chromaffin cells of the adrenal medulla. CART synthesised or secreted in the adrenal gland may regulate the catecholamine synthesis/secretion. Additionally, CART synthesised in the adrenal gland may be secreted into the blood.

**Cart and stress**

Studies show that various physical stress procedures such as water and food deprivation, exposure to cold, hypotension, hypertonic extracellular volume expansion, lipopolysaccharide treatment regulate CART expression in the brain\textsuperscript{48–52}.

Studies demonstrate that psychological stress also changes CART peptide and mRNA expression in the hypothalamus, limbic areas, brainstem and the adrenal gland. Forced swim stress (FSS) elevated CART peptide expression in the hypothalamus of male rats\textsuperscript{53,54}. In another study, FSS up-regulated CART mRNA expression in the DMH and down-regulated it in the ARC of male and female rats. CART mRNA expression was increased in the PVN only in female rats\textsuperscript{55}. In accordance with this data, FSS increased the number of CART-IR cells in the female PVN whereas no effect was observed in the male PVN\textsuperscript{56}. Two conclusions may be drawn based on these studies: First,
during acute stress, CART expression may be regulated differentially in distinct neural circuits regulating the HPA axis, sympatho-adrenal system and energy homeostasis. Second, CART expression during the stress response is sex-specific. Indeed, studies report that sex hormones regulate hypothalamic CART expression. In addition to the hypothalamus, FSS increased CART peptides in the frontal cortex, amygdala, medulla oblongata and the adrenal gland and CART mRNA in the central nucleus of amygdala (CeA) and locus ceruleus. Additionally, Hunter et al. showed that acute restraint stress increased CART mRNA in the CeA.

It seems likely that during acute stress, CART expression increases in hypothalamic nuclei, limbic brain regions and brainstem areas which regulate the activity of the PVN CRH neurons. CART released in the PVN may induce CRH synthesis/secretion, which in turn, will trigger the activity in the HPA axis and elevate CORT in the blood. Studies show that CORT, in turn, regulates CART expression in the hypothalamus. Increased CART synthesis/release in the hypothalamic and brainstem nuclei may also activate sympathetic activity and increase heart rate and blood pressure.

Alternatively, chronic social isolation decreased CART-IR cells and fibres in the PVN, ARC and locus ceruleus whereas it increased CART-IR fibres in the CeA. In another study, chronic mild stress decreased CART mRNA in the frontal cortex. Also, chronic restraint stress increased CART mRNA in the hippocampal dentate gyrus. It seems likely that chronic stress may cause habituation of CART signalling in specific brain regions.

CART and anxiety
CART may play a role in the neural circuits regulating anxiety. IVC CART treatment decreased the time spent in the open arms of elevated plus maze. This effect was reduced by anxiolytic drugs. Another study reported that during acute exposure to a cat, ICV and intra-CeA administration of CART potentiated the signs of anxiety in a social interaction test while the CART antibody prevented the predator-induced fear. Further, ICV and intra-CeA CART peptide administration for seven days prevented the habituation observed during chronic exposure to a cat and produced significant anxiety. During acute stress, increased CART signalling in the limbic brain regions may be an important component of the neural pathway producing anxiety and fear. However, during chronic stress, CART signalling may be down-regulated to produce habituation.

Conclusion
There is substantial literature to show that CART has significant effects on the HPA axis and sympatho-adrenal system activity. Although increasing number of studies report that CART expression is regulated by the stress response, the exact role of CART during the stress response appears to be complex and remains unclear. The existence of CART receptors has been demonstrated by binding and signaling studies. However, they have not yet been cloned. Characterisation of the CART receptors will improve our understanding of the role CART plays during the stress response and in stress-related diseases.

Abbreviations list
ARC, arcuate; CART, cocaine- and amphetamine-regulated transcript; CeA, central nucleus of amygdala; CRH, Corticotrophin releasing hormone; DMH, dorsomedial; dpPVN, dorsal parvicellular PVN; ELME, external layer of median eminence; FSS, forced swim stress; HPA, hypothalmo-pituitary-adrenal; IML, intermedio-lateral cell column; IR, immunoreactive; LHA, lateral hypothalamic; mpPVN, medial parvicellular subcompartment; NTS, nucleus of the solitary tract; PVN, paraventricular; RCA, retrochiasmatic area; RVL, rostral ventrolateral medulla; SON, supraoptic nucleus; SPN, sympathetic preganglionic neurons; vmpPVN, ventral part of medial parvicellular.

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