Cocaine- and Amphetamine-Regulated Transcript Peptides Play a Role in Drug Abuse and Are Potential Therapeutic Targets

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Michael J. Kuhar,[1] Jason N. Jaworski,[1] George W. Hubert,[1] Kelly B. Philpot,[1] and Geraldina Dominguez[1]

[1]Yerkes National Primate Research Center, Emory University, Atlanta, GA 30329

ABSTRACT

Cocaine- and amphetamine-regulated transcript (CART) peptides (55 to 102 and 62 to 102) are neurotransmitters with important roles in a number of physiologic processes. They have a role in drug abuse by virtue of the fact that they are modulators of mesolimbic function. Key findings supporting a role in drug abuse are as follows. First, high densities of CART-containing nerve terminals are localized in mesolimbic areas. Second, CART 55 to 102 blunts some of the behavioral effects of cocaine and dopamine (DA). This functional antagonism suggests that CART peptides be considered as targets for medications development. Third, CREB in the nucleus accumbens has been shown to have an opposing effect on cocaine self-administration. CREB may activate CART expression in that region, and, if so, CART may mediate at least some of the effects of CREB. Fourth, in addition to the effects of CART on DA, DA can influence CART in the accumbens. Thus a complex interacting circuitry likely exists. Fifth, in humans, CART is altered in the ventral tegmental area of cocaine overdose victims, and a mutation in the CART gene associates with alcoholism.

Overall, it is clear that there are functional interactions among CART, DA, and cocaine and that plausible cellular mechanisms exist to explain some of these actions. Future studies will clarify and extend these findings.

KEYWORDS: CART, cocaine, CREB, nucleus accumbens

INTRODUCTION

Cocaine- and amphetamine-regulated transcript (CART) peptides are peptide neurotransmitters and endocrine factors of physiologic importance. Several reviews have been written on various aspects of CART peptides.[1-4] This review focuses on the role of CART in drug abuse.

First, a note on nomenclature. Several fragments of pro-CART have been shown to be active;[5-7] two major fragments are rlCART 55 to 102 and rlCART 62 to 102, where "rl" refers to the long (l) form of the rat (r) transcript and its translated propeptide.[8] The identical peptides (except for one amino acid substitution at 55) in humans are written as hCART42-89 and hCART49-89, which reflects the finding that the human transcript is fully spliced to a smaller transcript yielding a shorter pro-CART of 89 amino acids compared with 102 for the rat long form (there is a rat short form of 89 amino acids as well). Most studies use rlCART 55 to 102 (or simply CART 55 to 102), which is commercially available, but other peptides have been examined, and we cannot rule out the idea that additional peptides may be shown to have activity as well.

The first demonstration of the existence of at least a fragment of CART peptide was in a publication by Spiess et al,[9] where the first 30 amino acids of CART 55 to 102 were sequenced and described as an "unknown" peptide with unknown function. In 1995, Douglass et al[10] reported that a transcript was increased in the striatum after acute cocaine or amphetamine administration (hence the name CART) and that the transcript coded for an apparent peptide neurotransmitter containing the 30 amino acid fragment of Spiess et al.[9] Additional key findings include the demonstration of CART peptide and its processing by Western blotting, immunoprecipitation, and sequencing;[6,11,12] its localization to neurons in the brain, gut, and other cells in the periphery;[13-23] its demonstrated electrophysiologic effects;[24-26] its ability to elicit expression of immediate early genes;[27] and its calcium-dependent release from hypothalamic explants.[28] A high-profile role for the CART peptide is its involvement in feeding; after noting the distribution of CART in the brain, our laboratory was the first to propose that CART regulates feeding,[13] and this was quickly confirmed using CART antibodies[29-31] and peptides.[31]

CART IN DRUG ABUSE: A MODULATOR OF MESOLIMBIC NEURONS AND PSYCHOSTIMULANTS

Douglass et al[10] and others[32,33] reported that CART mRNA increased in the striatum after acute or binge administration of cocaine or amphetamine. This was an impetus to examine CART in the context of drug abuse. However, it has now become clear that this finding by Douglass et al[10] is not always easily reproduced, and some have published negative data.[34,35] Nevertheless, despite these conflicting

Corresponding Author: Michael J. Kuhar, Yerkes National Primate Research Center of Emory University, 954 Gatewood Rd NE, Atlanta, GA 30329; Tel: (404) 727-1737; Fax: (404) 727-3278; E-mail: michael.kuhar@emory.edu

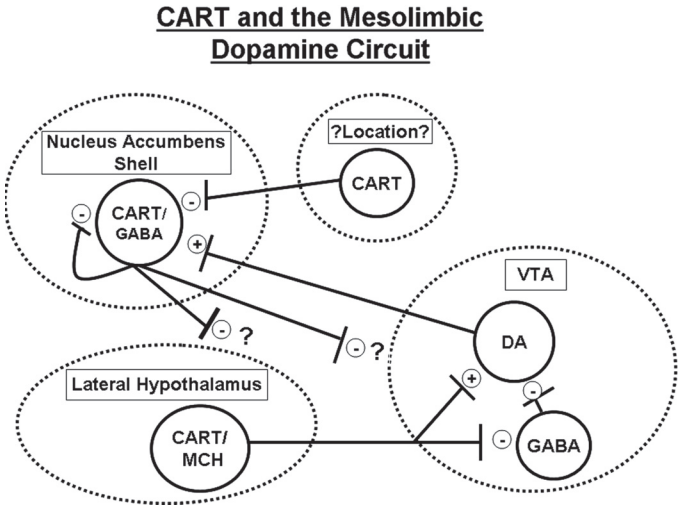

Figure 1. Mesolimbic circuitry and the influence of CART peptide. High densities of CART-containing nerve terminals are found in the VTA, and many CART-containing cells and processes are found in the nucleus accumbens (see text). Many of the nerve terminals in the VTA derive from a CART input from the lateral hypothalamus, which may play a role in integrating food and drug reward. CART in the VTA can influence DA neurons directly or indirectly through gamma amino butyric acid neurons. In the accumbens, DA nerve terminals are found on CART neurons (GABAergic medium-spiny output neurons) suggesting that DA influences CART. CART synapses in the accumbens from recurrent collaterals or other sources additionally suggest that CART can influence accumbal output.

reports, there is strong evidence implicating CART peptides in drug abuse, particularly in modulating mesolimbic function. In some cases, CART can affect dopamine (DA), and in other situations DA influences CART. Accordingly, we have developed a model with CART integrated into the mesolimbic circuitry (Figure 1).

First, CART peptides are found in key brain regions associated with reward/reinforcement; these include the ventral tegmental area (VTA), ventral pallidum, amygdala, lateral hypothalamus, and nucleus accumbens.[14,15,33,36,37] In the accumbens, the peptides colocalize with gamma amino benzoic acid in medium spiny output neurons and receive a DA input.[14,15,38] In the VTA, CART nerve terminals are found in all of the DAergic subnuclei, and some CART-containing nerve terminals synapse on DA-containing neurons and others on gamma amino butyric acid-containing neurons.[14,36] A major source of input to the VTA is from the lateral hypothalamus. Thus, CART is anatomically positioned to influence reward and reinforcement.

Second, CART is not only present in animals, as CART mRNA levels were changed in the VTA of cocaine overdose victims.[39,40] This is evidence that cocaine affects CART in animals and also in humans and supports a role for CART-cocaine interactions in humans.

Third, injection of CART into mesolimbic regions has behavioral effects related to psychostimulants. Kimmel et al[41] showed that injection of the CART peptide into the VTA caused a small increase in locomotor activity (which was blocked by haloperidol) and promoted conditioned place preference, suggesting that CART had psychostimulant-like effects. Our recent observation that intra-VTA CART causes an increase in the release of DA in the accumbens supports this (Figure 2). But, the magnitude of the locomotor and DA release effects were small compared with those of cocaine, and we found recently that cotreatment of animals with both intra-VTA CART and systemic cocaine produced only partially additive effects. The additivity between CART and cocaine seemed to occur at lower concentrations of the drugs, but at higher doses, CART tended to oppose the locomotor activity induced by systemic cocaine (Jaworski et al, manuscript in preparation). This sounds like CART is a functional "partial agonist" in the VTA. Evidence supporting this has been found in the accumbens. When CART was injected into the nucleus accumbens, by itself there was no effect, but when combined with systemic cocaine or amphetamine, the injection of CART again blunted the locomotor increasing the effects of the drugs (Figure 3).[42,43] Also, the CART peptide blunted the locomotor-increasing effects of DA injected into the accumbens, suggesting a downstream effect on the actions of DA (Figure 4). Therefore, in either the VTA or the accumbens, CART may have some small psychostimulant-like effects, but then it tends to oppose the actions of psychostimulants at higher doses. Although speculative, it is possible that CART may, therefore, be homeostatic or restorative in that it tends to oppose the large changes

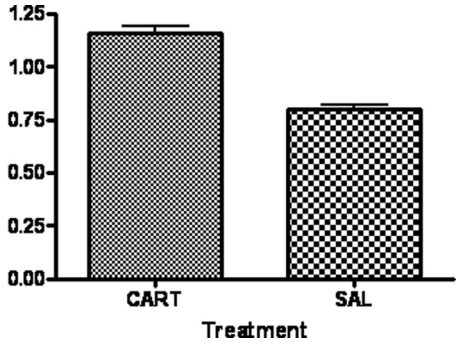

Figure 2. Intra-VTA injection of CART peptide produces a small increase in DA efflux in the nucleus accumbens. Because CART injected into the VTA has weak behavioral psychostimulant-like effects (see text), its potential to increase DA efflux by in vivo microdialysis was examined. DA efflux, summed over time, was indeed increased significantly ($P < 0.01$). This effect could be attributable to direct stimulation of DA neurons, by disinhibition of gamma amino butyric acid neurons (both of which receive CART inputs), or both (see Figure 1).

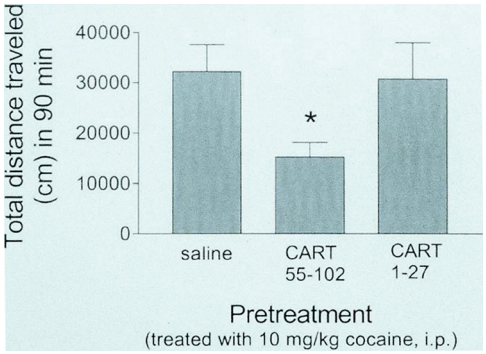

Figure 3. Intraaccumbal injections of CART peptide reduce the locomotor-activating effects of systemic cocaine. CART 55 to 102 injected into the accumbens minutes before i.p. injection of cocaine reduces locomotor activity. However, saline does not, and an inactive CART fragment, CART 1 to 27, does not. Data from Jaworski et al. [42]

caused by cocaine. A large number of controls for the active peptide and anatomic region were conducted in these experiments. CART, because it blunts at least some of the effects of psychostimulants, should be considered a target for medications development. The identification of the CART receptor would likely be essential for this.

Fourth, the intracerebroventricular injection of the CART peptide causes an increased turnover of DA in the nucleus accumbens.[44,45] This is an important functional counterpart of the observation that CART nerve terminals synapse on DA neurons in the VTA. Of course, the effect could be indirect, involving intervening neurons. Future experiments will be needed to resolve this.

Fifth, D3 DA receptors regulate the CART mRNA in accumbal cells.[2] D3 agonists deplete CART mRNA and the peptide in both the shell and the core (Hunter et al, manuscript in preparation). A reasonable hypothesis is that D3 receptors, which are well known to reduce cyclic adenosine monophosphate (cAMP) levels, could deplete CART by reducing active CREB in these cells. The effects of the D3 receptor are well known to interact with cocaine,[46-48] and our findings open the possibility that the D3 receptors may produce some of their effects through the CART peptides.

Sixth, other accumbal factors, such as cyclic AMP response element-binding protein (CREB), tend to have the same blunting effect on psychostimulants in that over-expression of CREB in the accumbens decreases the rewarding effects of cocaine.[49] It is relevant that CREB activates the promoter region of the CART gene[50-54] and may exert some of its effects through CART.

Seventh, whereas most genetic studies have focused on a connection between CART and human obesity, a recent study showed that a mutation in the CART gene was associated with alcoholism but not schizophrenia or bipolar disorder.[55] Again, CART is implicated in drug abuse not only in animals but also in humans.

Eighth, it has been shown that glucocorticoids are needed for cocaine to be self-administered.[56,57] We have found recently that corticosterone regulates CART levels in the nucleus accumbens. Perhaps CART somehow mediates the requirement for corticosterone.

In summary, DA, which is released/potentiated by drugs of abuse, can exert some control over CART peptide production and utilization, and CART peptides can, in turn, modulate mesolimbic DA function. The latter modulation can alter the effects of drugs of abuse. This hypothesized model (Figure 1 and 5) is under current exploration in our laboratory from several viewpoints.

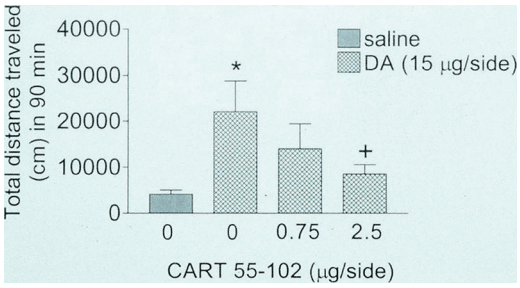

Figure 4. CART blunts locomotor activity induced by intraaccumbal injection of DA. Direct injection of DA into the nucleus accumbens increases locomotor activity, but that is reduced when CART peptide is coinjected with the DA. Thus, CART can reduce the direct effects of DA, suggesting that it is blunting the actions of DA rather than its release. This effect of CART is dose-responsive. Data from Jaworski et al. [42]

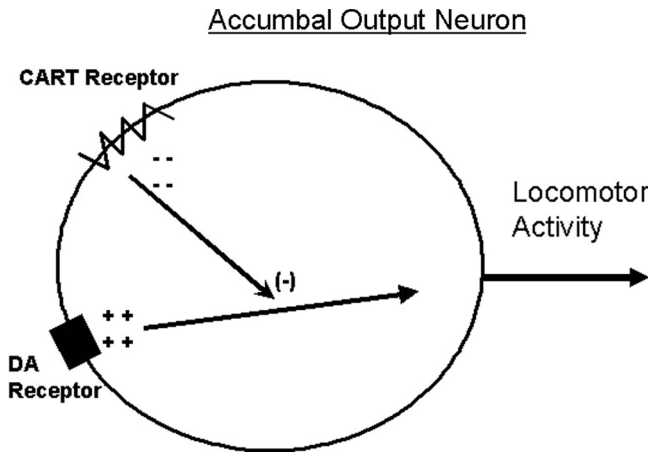

Figure 5. Cell model of how CART peptide could blunt the effect of DA in the accumbens. DA receptor activation in the nucleus accumbens can increase locomotor activity. CART peptides by themselves have no effect but can reduce the effect of DA, perhaps through an intracellular mechanism. The exact intracellular change that occurs is not yet known.

CART IS INVOLVED IN OTHER PHYSIOLOGIC PROCESSES

All of the evidence for this is too extensive to be detailed here, but brief summaries can give the impressive picture of the importance of these peptides. This is an interesting example of how research in drug abuse can impact other areas.

In feeding, CART appears to play a major role in determining body weight in humans (reviewed in Ref.[4]). The human genetic data are very strong, and the animal studies are very supportive. A mutation in the human CART promoter causes increased girth.[58] Another mutation in the reading frame that appears to interfere with processing, sorting, and trafficking results in increased body weight.[59,60] Cells expressing the latter mutation produce altered levels of CART peptide; thus, the mutation has an actual effect on peptide levels.[52] Also, CART knockout mice exhibit a tendency to gain weight.[61] Because of the likely overlap of feeding reward mechanisms and drug reward mechanisms, studies of feeding mechanisms are likely to have some significance for drug abuse as well.

Stress is a risk factor for drug abuse, and CART peptides appear involved in stress. Hypothalamic CART mRNA and peptides are regulated by glucocorticoids,[62,63] and stress alters the levels of CART peptides in the arcuate nuclei (Balkan, manuscript in preparation). The blood levels of CART are also at least partially affected by glucocorticoids.[23] Also, injection of CART 55 to 102 into the cerebral ventricles induces fos expression in cells in the paraventricular nucleus that contain CRF, suggesting that CART is involved in controlling the release of corticotropin-releasing factor (CRF).[64,65] Also, the CART promoter contains regulatory elements that could be involved in regulation by glucocorticoids as a result of interactions with transcription factors.[50] However, the precise and complete role of CART in stress is not yet fully known and is under current investigation. Of course, in this context, it is also important to note that glucocorticoids are critical factors for cocaine self-administration,[56,57] and the role of glucocorticoids in CART regulation is, therefore, important.

The CART peptide has antinociceptive effects,[66-68] although the precise nature of these effects is being clarified. In endocrine regulation, evidence for a role for CART is increasing. CART is found in the nuclei and regions historically associated with endocrine control, and CART peptides are found in the portal blood.[69] CART also caused changes in prolactin release and inhibited the release of thyroid-stimulating hormone from pituitary cells in culture.[70-72]

In development, CART has been found early in the brain and gut, as well as in the ovary and pancreas,[17,19-22,73] suggesting a role in development. CART has been shown to be neurotrophic in in vitro cell culture systems.[5] These are open areas of research where little has been published, but much is ongoing.

CART IS A REGULATED mRNA AND PEPTIDE

Whether or not CART is regulated or simply produced constitutively is relevant. The peptides involved in physiologic regulation and signaling are routinely "regulated, " that is, their synthesis is controlled by various physiologic factors. The CART levels are known to change fairly rapidly in a couple of hours in response to various stimuli. First, there was the initial observation that CART mRNA increased 1 hour after the acute injection of cocaine or amphetamine.[10] Although this has been repeated by some,[32,33] it has been challenged by others.[34,35] In our laboratory, we tend to get increases in CART mRNA levels, but only after high-binge doses of cocaine (data unpublished). Nevertheless, a regulation seems to occur at least under some conditions. Second, the D3 DA receptor agonists decrease the CART mRNA levels in the nucleus accumbens in a matter of hours, and this is blocked by D3 antagonists. In D3 knockout mice, the CART mRNA levels are up regulated in the accumbens.[74] Third, CART levels in the nucleus accumbens and in other regions undergo diurnal variations (Vicentic et al, in press); this and perhaps other rhythms may somehow cause or reflect the diurnal variation in cocaine intake.[75] Fourth, leptin has been shown to regulate CART mRNA in specific nuclei in the hypothalamus,[31,76] and the CART proximal promoter region contains a consensus signal transducer and activator of transcription site, which can be activated via leptin signaling.[50,54] Fifth, glucocorticoids, which can regulate drug intake,[56,57] also regulate CART levels in the peripheral blood[23,62,63] and in the brain.[62,63] Sixth, several studies in our laboratory and elsewhere show that changes in cAMP levels activate CREB, which, in turn, activates the CART gene,[50-54] and CREB plays a role in drug abuse.[49]

Whereas changes in levels may not always reflect transcriptional regulation, we assume that many of the above observations do indeed reflect it. Understanding the mechanisms regulating the CART promoter is key to understanding how CART is regulated and influenced. In several studies, we found that the CART proximal promoter contains a consensus cyclic adenosine monophosphate response element site that is functional in both GH3 (neuroendocrine-derived cell line) and CATH.a cells (neuronal-derived cell line) and is involved in CREB-mediated transcription.[50-54]

CATH.a cells are a neuronally derived cell line and express the type 1 CRF receptor (CRF-R1).[77] They are derived from locus coeruleus neurons, which are known to express CART.[13] CATH.a cells were transfected with −641 CART-LUC (a construct containing the proximal promoter

region of the CART gene and the luciferase gene as a marker for promoter activity) and incubated in the presence of CRF alone or with combinations of H89 (an inhibitor of protein-kinase A) and CP154,526 (a selective antagonist to the CRF-R1). Luciferase expression was increased after CRF treatment, and the increase was reduced by H89. The response was CRF specific, because CP154,526 caused a significant reduction of luciferase activity after CRF treatment. Taken together, these data suggest that a naturally occurring peptide neurotransmitter (CRF) can influence CART expression in CATH.a cells and supports the hypothesis that CART transcriptional regulation occurs in neurons via the camp/protein kinase A pathway.[52] The utilization of a promoter-luciferase construct demonstrates true transcriptional regulation rather than simply showing a change in mRNA levels.

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