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The CART (Cocaine- and Amphetamine-Regulated Transcript) System in Appetite and Drug Addiction

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ABSTRACT

CART (cocaine- and amphetamine-regulated transcript) peptides are neuromodulators that are involved in feeding, drug reward, stress, cardiovascular function, and bone remodeling. CART peptides are abundant but discretely distributed in the brain, pituitary and adrenal glands, pancreas, and gut. High expression of CART in discrete hypothalamic nuclei associated with feeding has led to behavioral and pharmacological studies that strongly support an anorectic action of CART in feeding. Subsequent studies on humans and transgenic animals provide additional evidence that CART is important in the regulation of appetite as mutations in the CART gene are linked to eating disorders, including obesity and anorexia. The expression of CART in the mesolimbic dopamine circuit has lead to functional studies demonstrating CART's psychostimulant-like effects on locomotor activity and conditioned place preference in rats. These and other findings demonstrated that CART modulates mesolimbic dopamine systems and affects psychostimulant-induced reward and reinforcing behaviors. The link between CART and psychostimulants was substantiated by demonstrating alterations of the CART system in human cocaine addicts. CART seems to regulate the mesolimbic dopamine system, which serves as a common mechanism of action for both feeding and addiction. Indeed, recent studies that demonstrated CART projections from specific hypothalamic areas associated with feeding to specific mesolimbic areas linked to reward/motivation behaviors provide evidence that CART may be an important connection between food- and drug-related rewards. Given the enormous public health burden of both obesity and drug addiction, future studies exploring the pharmacotherapies targeting CART peptide represent an exciting and challenging research area.

CART (cocaine- and amphetamine-regulated transcript) is a peptide that functions as both a central and peripheral neurotransmitter. The CART peptide was first sequenced as a peptide with unknown function (Spiess and Vale, 1980) and then confirmed in a study reporting that a protein product of an mRNA up-regulated in the ventral striatum following acute administration of cocaine and amphetamine (Douglass et al., 1995) was the peptide described by Spiess and Vale (1980). The human propeptide form of CART is processed into two biologically active fragments, 42 to 89 and 49 to 89,

which correspond to rat 55 to 102 and 62 to 102 fragments, respectively. Although physical description, synthesis, and processing of the peptides have been well studied (Kuhar et al., 2000), very little is known about their metabolism. CART has been implicated in a variety of physiological processes, including feeding, bone remodeling, sensory processing, endocrine regulation, stress and anxiety, and the rewarding and reinforcing properties of psychostimulants (Hunter and Kuhar, 2003). Moreover, CART was shown to exert effects on neuronal activity, such as *N*-methyl-D-aspartate receptor-mediated neurotransmission (Hsun Lin et al., 2005) and ventromedial hypothalamic nucleus (VMN), dorsal raphe nucleus, and paraventricular nucleus (PVN) hypothalamic neurons (Davidowa et al., 2005), suggesting that it may play a part in the neuroplasticity of the hypothalamic feeding

ABBREVIATIONS: CART, cocaine- and amphetamine-regulated transcript; VMN, ventromedial hypothalamic nucleus; LH, lateral hypothalamus; Arc, arcuate nucleus; PVN, paraventricular nucleus; NTS, the nucleus of the solitary tract; CRF, corticotrophin-releasing factor; DMN, dorsomedial nucleus of the hypothalamus; NPY, neuropeptide Y; CTA, conditioned taste aversion; DA, dopamine; VTA, ventral tegmental area; NAc, nucleus accumbens; GABA, γ-aminobutyric acid; LMA, locomotor activity; CREB, cAMP-response element-binding protein; KO, knockout.

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circuits. The physiological importance of CART was further substantiated in numerous human studies demonstrating a role of CART in both feeding and psychostimulant addiction. For instance, various polymorphisms in the CART gene affect multiple facets of feeding (Hunter and Kuhar, 2003), and CART mRNA levels are increased in the brain of human cocaine overdose victims (Tang et al., 2003). Evidence of CART involvement in feeding and psychostimulant-induced reward and reinforcement is drawn from a large number of studies, and this review will focus on the steadily accumulating research indicating an important modulatory role for CART peptide in the rewarding/reinforcing properties of both natural (food) and unnatural (drugs) stimuli.

Feeding and Obesity

Anatomical Evidence of CART's Role in Feeding

Douglass et al. (1995) observed that the distribution pattern of a novel mRNA up-regulated by cocaine and amphetamine was predominantly confined to hypothalamic neuroendocrine neurons and limbic circuits important in reward. Koylu et al. (1997) initially suggested that CART plays a role in food intake. Subsequent studies supported this contention as CART immunoreactivity was found in brain areas relevant to feeding (Elmquist et al., 1999), including the VMN, lateral hypothalamus (LH), arcuate nucleus (Arc), PVN, the nucleus of the solitary tract (NTS) (Koylu et al., 1998), and the nucleus accumbens (NAc) (Smith et al., 1999).

In addition to its distribution in feeding-associated brain regions, CART colocalizes with peptides that regulate feeding. For example, in the PVN, CART is coexpressed with thyrotropin-releasing hormone (Broberger, 1999) and corticotrophin-releasing factor (Sarkar et al., 2004), which suggests its involvement in the regulation of pituitary hormone secretion and/or energy metabolism. CART is coexpressed with melanin-concentrating hormone within the dorsomedial nucleus (DMN) and LH and pro-opiomelanocortin in the Arc (Vrang et al., 1999a). CART interacts with two most important mediators of feeding, NPY (Lambert et al., 1998) and leptin; however, it colocalizes only with c-*fos* in leptin-activated neurons (Elias et al., 1998) of the DMN and LH nuclei but not with NPY in the Arc nucleus (Vrang et al., 1999a).

Hypothalamic CART is also coexpressed with cannabinoid CB1 receptors that regulate feeding behavior and energy homeostasis (Cota et al., 2003). This interaction may be important for the regulation of appetite, because CB1 mice with a disrupted CB1 gene are lean and hypophagic and exhibit attenuated levels of CART mRNA. The findings that CART is colocalized with both orexigenic and anorexigenic peptides suggest that CART's role in appetite control and energy homeostasis is most probably modulatory.

Outside the hypothalamus, it is postulated that CART exerts its anorectic action in discrete areas of the hindbrain, including the NTS (Broberger et al., 1999). CART immunoreactivity was found in the vagus nerve and in the viscerosensory nodose ganglion. In the vagus nerve, CART terminals originate from various nuclei, including the NTS. In the NTS, a large proportion of CART-labeled neurons express cholecystokinin A receptor mRNA (Broberger et al., 1999). Because cholecystokinin induces satiety, CART's action in feeding may be mediated, in part, via postprandial satiety effects of cholecystokinin.

In addition to its widespread central localization, CART is abundantly present in peripheral areas associated with feeding. For example, CART is present in rat ileum (Couceyro et al., 1998) and numerous myenteric neurones throughout the gastrointestinal tract (Ekblad et al., 2003). CART is also expressed in several islet cell types during rat development and is up-regulated in β-cells of diabetic rats, suggesting its importance for the regulation of islet hormonal secretion (Wierup et al., 2006). Finally, CART is selectively expressed in a population of sympathetic preganglionic neurons and adrenal glands, supporting its potential role as a signaling molecule in the sympatho-adrenal axis (Dun et al., 2000). The areas where CART may exert its anorectic properties are depicted in Fig. 1.

Functional Evidence of CART's Role in Feeding

Initial findings have demonstrated that i.c.v. administration of CART peptide profoundly inhibited feeding in rats, which was still apparent following NPY-stimulated feeding (Lambert et al., 1998). Administration of a CART antibody stimulated feeding, suggesting that endogenous CART peptide exerts an inhibitory tone on feeding (Kristensen et al.,
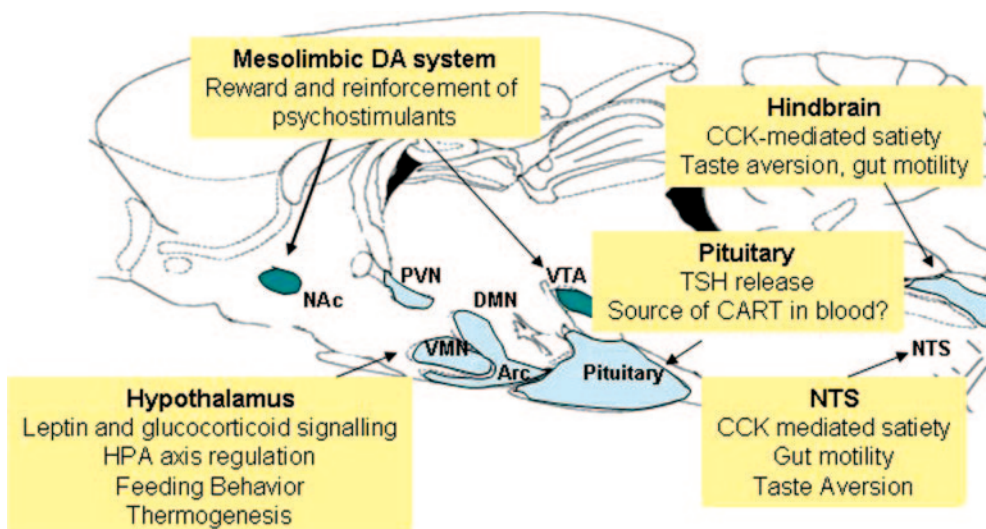

Fig. 1. Anatomical distribution of CART mRNA and peptide in brain regions associated with feeding, appetite control, and the rewarding effects of psychostimulants. The four major regions/pathways are the mesolimbic DA system (VTA and NAc), several nuclei of the hypothalamus (PVN, DMN, VMN, and Arc), the pituitary, and the hindbrain (NTS). Regions in blue are associated with feeding and appetite regulation, and although the mesolimbic DA pathway (green) is strongly associated with the rewarding effects of psychostimulants, it also appears to play a role in appetite. Figure is adapted and modified from Hunter et al. (2004) with permission.

1998; Lambert et al., 1998). Furthermore, administration of CART peptide activates an early gene, *c-fos*, in several hypothalamic and brain stem nuclei involved in feeding and metabolism, further implicating these regions in CART's anorectic action (Vrang et al., 1999b). Chronic central infusions of CART inhibited feeding and body weight gain in both lean and obese animals (Larsen et al., 2000). In addition, i.c.v administration of CART was accompanied by decreases in plasma insulin and leptin levels and increases in lipid oxidation, both of which limit fat storage (Rohner-Jeanrenaud et al., 2002).

In addition to inhibiting feeding, i.c.v. administration of CART peptide produced disruption of locomotor activity, raising questions about the specificity of its anorectic effects (Abbott et al., 2001; Aja et al., 2002). For instance, administration of CART peptide into either the third or fourth ventricle decreased intake of a liquid sucrose diet, altered licking patterns, and produced movement-associated tremors in rats. The rapidity by which CART caused these hypophagic and motor effects suggests a periventricular site of action, possibly via sites in the caudal hindbrain (Aja et al., 2001). Injections of CART peptide into the fourth ventricle also produced conditioned taste aversion (CTA) accompanied by reduced ingestion of both water and sweet liquid diets (Aja et al., 2002). This suggests that the ability of CART to reduce food intake when administered into the fourth ventricle may be secondary to the production of an aversive state. The mechanisms of CART-induced CTA are not clear; CART could mimic signals of gastrointestinal malaise, or alternatively, it may be a signal that is integral in developing CTA (Aja et al., 2002). Another mechanism for CART's anorectic effect may be via activation of the sympathoadrenal system as administration of CART peptide increases blood glucose levels (Vrang et al., 2000) and blood pressure (Matsumura et al., 2001). Elucidation of the exact mechanisms involved in CART's anorectic effects and resolving these issues will have a significant impact on the development of the CART system as a pharmacological target for appetite control and body weight.

Whereas numerous studies have reported attenuation of feeding following i.c.v. injections of CART, studies investigating the impact of intranuclear CART injections on feeding have been inconclusive. For example, direct administration of CART peptide into the NAc shell inhibits feeding without affecting motor behavior (Yang et al., 2005). In contrast, direct administration of high doses of CART into several discrete hypothalamic nuclei increased food intake (Abbott et al., 2001). This effect was not apparent until 1 to 2 h following administration and was accompanied by a significant decrease in feeding that occurred 4 to 24 h postinjection, perhaps suggesting either i) a compensatory decrease in feeding or ii) a delayed anorectic effect following the initial stimulation of feeding. Likewise, after gene transfer that elevated CART peptide levels in the Arc, animals consumed more food and their body weight increased (Kong et al., 2003). Moreover, this study showed a strong correlation between CART levels and thermogenesis; overexpression of the CART gene in the rat Arc combined with sustained exposure to cold increased CART mRNA levels and weight loss, suggesting an increase in energy expenditure and a potential role of CART in cold adaptation and cold-induced hyperphagia in rodents. These studies received support from a report showing that self-starved anorexic (*anx/anx*) mice display low body weight and marked behavioral alterations, including abnormal movement and hyperactivity (Johansen et al., 2000). These abnormalities were accompanied by down-regulated CART mRNA and peptide levels in the Arc, which could be interpreted as a protective mechanism of suppressing the anorexigenic state in catabolic animals (Johansen et al., 2000). In conclusion, CART-mediated regulation of appetite in the hypothalamus is a complex process, and it is plausible that the hypothalamic CART system is differentially regulated. Differential responses to i.c.v. versus intranuclear administration of CART suggests that CART may be activating two independent appetite circuits within the hypothalamus, one that is anorexigenic and the other that is orexigenic.

It is currently unknown whether peripheral CART plays a role in feeding. Fasting alters CART blood levels and abolishes its diurnal rhythm (Vicentic, 2006), suggesting that peripheral CART levels are sensitive to fuel availability. However, direct evidence of the involvement of peripheral CART in feeding behavior is still lacking; intraperitoneal administration of CART peptide did not affect feeding (Jensen et al., 1999), whereas mice lacking the CART gene displayed increased body weight and impaired glucose elimination and insulin secretion but no difference in food intake (Wierup et al., 2006).

Functional Responses of CART to Different Metabolic and Feeding States

The effects of centrally administered CART peptide on metabolism and associated behaviors are not limited to feeding. For example, i.c.v. administration of CART reduces gastric motility, emptying, and secretion (Asakawa et al., 2001). Research conducted in mutant animals also supports the role of CART as a mediator of metabolic regulation. Compared with wild type, CART knockout mice are prone to being obese and are susceptible to high-fat diet-induced obesity (Asnicar et al., 2001). Genetic deletion of CART impairs glucose-stimulated insulin secretion, suggesting that CART is needed for normal pancreatic function. Furthermore, CART peptide levels were shown to respond to changes in the metabolic state of normal, wild-type animals. Fasting-induced decreases in CART levels were also observed in several studies (Bertile et al., 2003; Yang et al., 2005; Vicentic, 2006). These studies suggest that CART-producing neurons are involved in energy homeostasis and that decreases in CART levels following acute dietary restriction may reflect a compensatory mechanism to reduce caloric expenditure. In contrast, a high-energy diet acutely up-regulated hypothalamic CART mRNA (Wortley et al., 2004), which was positively correlated with a reduction in lipid storage, promotion of fat utilization, and circulating leptin levels. Obese mice with hyperleptinemia also display up-regulation of CART expression (Tsuruta et al., 2002). These findings suggest that activation of CART neurons stimulates catabolic pathways that may minimize excessive accumulation of body fat under conditions of positive energy balance.

Contradictory results have been reported following chronic exposure to high-fat diets. CART mRNA and peptide expression was decreased in the Arc according to one report (Tian et al., 2004) and increased in another (Wortley et al., 2004). Moreover, hyperthyroidism, which is accompanied by increased food intake, decreases CART expression in the PVN

(Lopez et al., 2002). Discrepancies in findings may be due to different paradigms used, different diets, and/or different periods of exposure to each diet. Indeed, neurons of the hypothalamic regulatory system controlling body weight differentially react to CART (Davidowa et al., 2005). This effect is dependent on the nutritional state of the animal (i.e., overweight, fed, or short-term fasting), suggesting that increased inhibitory responses to CART may reflect a general mechanism in adaptation of neuronal regulatory systems to the nutritional state.

Interaction of CART with Leptin

CART's anorectic action is strongly associated with that of peripheral adiposity signal and one of the most important regulators of food intake, leptin. CART mRNA levels are low in various hypothalamic nuclei of rodents with a disrupted leptin system (Murphy, 2005). Leptin replacement subsequently restores CART transcript levels in ob/ob mice that lack functional leptin receptors (Kristensen et al., 1998), and chronic leptin injections decrease body weight and adiposity and increase CART expression in mice (Ahima and Hileman, 2000). In a mouse model of anorexia (anx/anx) with disrupted leptin signaling and reduced food intake, CART levels are markedly decreased (Johansen et al., 2000). Furthermore, in a polygenic model of high-fat diet/energy-induced obesity accompanied by hyperleptinemia and diabetes, CART levels are attenuated in the Arc nucleus because of a defect in central leptin signaling and receptors (Tian et al., 2004). These results are not surprising because CART proximal promoter has a signal transducer and activator of transcription binding site that may be relevant for the effects of leptin on CART expression (Dominguez et al., 2002).

Interaction of CART with Glucocorticoids

It is well known that an excess of glucocorticoids is associated with obesity in humans and animals, especially with abdominal obesity (Dallman et al., 2004). A regulatory effect of glucocorticoids on CART expression was demonstrated in several central and peripheral tissues (Vicentic, 2006). CART induced c-*fos* expression in corticotrophine-releasing peptide neurons in the PVN may induce corticosterone secretion, thus potentially contributing to the inhibitory effects of CART on feeding (Larsen et al., 2000). The effect of glucocorticoids on CART may be direct because corticosterone increases CART levels, whereas metyrapone abolishes the evening peak in CART levels (Vicentic, 2006). It is also of interest that CART levels in blood exhibit a circadian rhythm that is similar to that of glucocorticoids and that it is altered by adrenalectomy (Vicentic, 2006). These results suggest that CART peptide plays a role in the function of hypothalamic-pituitary-adrenal axis activity, which may be relevant to its action in feeding.

CART and Human Obesity

The human CART gene is ideally positioned as a candidate for obesity, because it maps to chromosome 5q13-14, which is a susceptibility locus for obesity (Hager et al., 1998; Echwald et al., 1999). Polymorphisms of the CART gene, as well as differences in CART peptide levels, have been implicated in hereditary obesity, variations in energy expenditure, hip-to-waste size ratio, overindulgent forms of ingestive behavior, and anorexia nervosa (Challis et al., 2000; del Giudice et al., 2001; Yamada et al., 2002; Guerardel et al., 2005).

Some of the most convincing evidence supporting a role for CART in human obesity was described in a study on an obese Italian family (del Giudice et al., 2001). A missense mutation in CART gene resulted in a substitution of Leu with Phe and was linked to severe early-onset obesity over three generations. Interestingly, the same mutation in a CART cDNA construct was found to alter CART peptide levels in AtT20 cells, possibly through CART processing, sorting, and trafficking (Dominguez et al., 2004). The mutation and phenotype described indicate that the CART system plays an important role in the regulation of human body weight and possibly carry further health implications. Indeed, members of the same Italian family carrying the L34F mutation exhibited higher depression and anxiety

TABLE 1

Possible mechanisms of CART's regulation of body weight

Although there is no conclusive evidence delineating the mechanisms of CART's anorexigenic action, the following hypotheses have been suggested.

Increasing Thermogenesis and Energy Expenditure
- Leptin activates POMC/CART neurons that innervate sympathetic preganglionic neurons in the thoracic spinal cord (Elias et al., 1998).
- Leptin and CART levels are decreased in arcuate nucleus in animal models of anorexia (Johansen et al., 2000).
- CART-positive terminals in the intermediolateral cell column and other sympathetic autonomic nuclei (Koylu et al., 1998; Dun et al., 2000) synapse with preganglionic intermediolateral cell column neurons.
- Repeated intranuclear injections of CART into the Arc increases thermogenic responses to BRL 35135 (Kong et al., 2003).
- Genetic disruption of the CART gene is associated with reduced resting energy expenditure and is linked to obesity in more than one generation of an Italian family (del Giudice et al., 2001).

Mediation of Postprandial Satiety Effects of Cholecystokinin
- CART is expressed in vagal afferent neurons expressing cholecystokinin a (CCKa) receptors sensitive to cholecystokinin (Broberger et al., 1999). Food intake induces CCK release in the gut, which stimulates CCKa receptor expression. This suggests that CART may terminate feeding by acting as a mediator released from central vagal afferents after CCKa receptor stimulation.

Decreasing Rate of Gastric Emptying
- Injections of CART into the fourth ventricle decrease the rate of gastric emptying (Asakawa et al., 2001) CRF and cholecystokinin also reduce food intake and inhibit gastric emptying, and CART induces c-*fos* expression in CRF containing neurons in the PVN.

Regulation of Motor Pattern Generators That Controls Ingestion
- Injections of CART into fourth ventricle reduces liquid food and water intake causing tremors and conditioned taste aversion that may interfere with normal ingestion (Aja et al., 2001; Aja et al., 2002). Indeed, CART increases c-*fos* immunoreactivity in the nucleus solitary tract and dorsolateral parabrachial nucleus (Vrang et al., 1999b), supporting an action of CART within the brain stem and mechanisms involving vagal satiety signals.

Other Issues
- Centrally administered CART augments circulating nonesterified fatty acid levels and decreases lipoprotein lipase activity in adipose tissue in rats on a high-fat diet (Wortley et al., 2004), suggesting a mechanism of decreasing lipid storage and stimulation of lipid use.
- CART variants with melanocortin-4 receptor mutations results in the propensity to be sedentary. Recent identification of specific CART receptor binding and signaling (Vicentic et al., 2006) suggest the eminent identification of a CART receptor

scores compared with controls, thus suggesting that CART may link feeding and emotion (Yanik et al., 2006). Another finding linking CART to human obesity demonstrated six polymorphisms in the CART gene promoter region in thirty obese Japanese men (Yamada et al., 2002). In addition, a deletion mutation in exon 3 of the CART gene may be linked to augmented total cholesterol levels in a population of patients suffering from type 2 diabetes (Fu et al., 2002). Future studies examining CART gene polymorphisms would contribute to the understanding of obesity and may potentially lead to the use of CART peptide as a therapeutic target for appetite, affective disorders, and any related secondary complications, including cardiovascular disease. Table 1 summarizes possible mechanisms of CART's regulation of body weight.

Drugs of Abuse

Neuroanatomical Evidence for CART's Role in the Actions of Psychostimulants

A role for CART in modulating the reward and reinforcing effects of psychostimulants is suggested by a variety of studies. First, CART mRNA and peptide are distributed in the majority of reward-associated regions. Second, CART-containing neurons form synapses with neurons containing neurotransmitters associated with addiction, including dopamine (DA) and GABA. Finally, colocalization studies indicate that CART is expressed in neurons that synthesize a variety of neurochemicals implicated in the pharmacological actions of psychostimulants. Perhaps two of the most important regions pertinent to CART's role in drug-mediated reward, reinforcement, and behavior are part of the mesolimbic DA pathway, the ventral tegmental area (VTA), and NAc. In the VTA, nerve terminals containing CART mRNA and peptide are associated with both DA- and GABA-synthesizing neurons (Koylu et al., 1999; Dallvechia-Adams et al., 2002), whereas in the NAc, CART is preferentially localized to the shell region of the accumbens, which is highly sensitive to the effects of drugs of abuse (Hurd and Fagergren, 2000). Two other regions, the amygdala and cortex, play important roles in both the acute reinforcing effects and long-term consequences of drug use. CART mRNA is expressed in several regions of the extended amygdala (Hurd and Fagergren, 2000). Moreover, in a functional sense, binge cocaine administration up-regulates CART mRNA in the amygdalae, suggesting that the rewarding effects of cocaine may be mediated via this CART system (Fagergren and Hurd, 1999). Because of the role of amygdala in mediating environmental stressors, CART may associate otherwise neutral environmental stimuli with the presence of drugs. The cortex is activated by environmental cues that motivate drug intake and is strongly associated with the cognitive effects of long-term drug abuse. CART mRNA and peptide expression vary dramatically throughout the cortex (Couceyro et al., 1998), with significant differences noted in cortical CART mRNA expression between rats and humans. In humans, the highest levels of CART mRNA are found in discreet regions of the cortex (Couceyro et al., 1997) associated with the motivational process of drug use, which supports a potential role for CART in the reward. In contrast, rat expression of cortical CART mRNA is mostly limited to discreet areas in the primary somatosensory cortex (Couceyro et al., 1997), which is more indicative for a role of CART in sensory processing.

Neurons containing CART mRNA and/or peptide synapse with other neurons containing neurochemicals were involved in drug addiction (Vrang et al., 2000; Dallvechia-Adams et al., 2002), perhaps most importantly DA and GABA. Specifically, CART nerve terminals in the VTA contact both dopaminergic neurons, which project to the accumbens and GABAergic interneurons (Dallvechia-Adams et al., 2002). Thus, CART may modulate DA activity in the VTA through either i) the direct stimulation of dopaminergic neurons or ii) the indirect disinhibition of DA neurons via CART-mediated inhibition of GABA release. Conversely, in the NAc shell, dopaminergic terminals synapse with CART peptide-containing GABAergic medium spiny output neurons, suggesting that accumbal DA may modulate CART peptide activity (Koylu et al., 1999). Therefore, a functional relationship among CART peptide, DA, and GABA can be envisioned in the reinforcing effects of psychostimulants.

Colocalization studies also support a role for CART in the actions of psychostimulants. For instance, in the NAc, CART peptide colocalizes with dynorphin and GABA (Dallvechia-Adams et al., 2002), which have been linked to drugs of abuse (Fagergren and Hurd, 1999; Roberts, 2005). The majority of CART peptide-immunoreactive terminals identified in the NAc appear to be axon collaterals of striatal projection neurons that express GABA (Smith et al., 1999). Furthermore, CART colocalizes with GABA in axon terminals of the VTA (Dallvechia-Adams et al., 2002), suggesting that CART and GABA may act as cotransmitters in accumbal projection neurons involved in the modulation of the effects of psychostimulants. CART also colocalizes with tyrosine hydroxylase in axon terminals of VTA neurons (Koylu et al., 1999), indicating a probable modulatory relationship between CART and DA. Taken together, the anatomical evidence demonstrates that CART plays an important role in modulating the brain circuitry involved in psychostimulant addiction, including the acute reinforcing actions and the body's long-term response to psychostimulants.

Neuropharmacological Evidence of a Role for CART in Psychostimulant Addiction

The relationship between CART and other neurotransmitters linked to the actions of psychostimulants is complex. For instance, i) under certain conditions, CART mRNA and peptide levels are directly affected by cocaine and amphetamine, ii) CART peptide has an affect on (and is affected by) DA and GABA, and iii) the regulation of the CART gene involves CREB, which has been implicated in psychostimulant addiction (Table 1).

CART's involvement in the actions of psychostimulants was first noted in a study demonstrating that acute cocaine and amphetamine up-regulated CART mRNA in the rat brain (Douglass et al., 1995). This report has proven controversial as this finding has been difficult to replicate (Vrang et al., 2002; Marie-Claire et al., 2003; Hunter et al., 2005). It now appears that a binge-dosing regime, rather than acute administration of cocaine, reliably increases CART expression (Fagergren and Hurd, 1999; Hunter et al., 2005). However, there seems to be at least two physiological conditions in which CART mRNA levels are increased by acute cocaine. First, cocaine seems to affect the regulation of the CART gene under conditions of enhanced cAMP signaling (Jones and

Kuhar, 2006). Second, CART mRNA and peptide levels are sensitive to stress as corticosterone increases accumbal CART mRNA, whereas pretreatment with metyrapone decreases CART peptide levels (Vicentic, 2006). Glucocorticoids also seem to be involved in the modulation of rewarding behaviors, including food and drug intake (Goeders, 2002). Interestingly, CART's diurnal rhythm correlates with daily variations in glucocorticoids levels (Vicentic, 2006); consequently, CART may influence the diurnal variation in cocaine sensitization and reward. Therefore, under certain conditions involving stress or increased cAMP, psychostimulants may alter CART mRNA and peptide levels.

The CART system modulates mesolimbic dopaminergic activity and attenuates the behavioral effects of cocaine and amphetamine (Jaworski et al., 2003; Kim et al., 2003), suggesting a complex relationship between CART, DA, and psychostimulants and the brain's reward/reinforcement pathways. Increasing evidence suggests that CART affects brain DA levels, and vice versa, DA seems to regulate CART peptide levels. For instance, CART peptide inhibits DA release in vitro (Brunetti et al., 2000). In contrast, central administration of CART increases DA turnover (Yang et al., 2005), whereas direct intra-VTA injections of CART moderately increase extracellular DA levels in the NAc (Kuhar et al., 2005). That being said, although central injections of CART increase the activity of the nigrostriatal and mesolimbic DA systems in the rat, this treatment does not alter dopaminergic activity in the prefrontal cortex (Shieh, 2003). This is important because humans and rodents have different patterns of cortical CART expression (Koylu et al., 1998). Consequently, potential differences in CART's function in the cortex, which could include effects related to psychostimulant abuse, may exist.

In addition to its effects on brain dopamine turnover, CART expression may be regulated via DA D3 receptors (Beaudry et al., 2004; Hunter et al., 2006). First, CART and DA receptor transcripts colocalize (Beaudry et al., 2004). Second, dopaminergic nerve terminals in the NAc synapse on CART-containing neurons (Koylu et al., 1999), hence providing the proximity required for neurotransmitter signaling. These studies suggest that DA plays a role in regulating CART gene expression possibly via the activation of CREB. Indeed, CART gene expression is regulated via cAMP signaling and pCREB in vivo as centrally administered forskolin activated cAMP, phosphorylated CREB, and increased CART mRNA and peptide levels (Jones and Kuhar, 2006). Moreover, cocaine potentiated forskolin-induced CART mRNA expression. In support, cocaine overdose victims have both increased pCREB and CART levels (Tang et al., 2003), suggesting an association between the drug, the transcription factor, and the neuropeptide. Thus, perhaps CART peptide levels are regulated by cocaine in a chronic abuser, whose basal cAMP signaling is enhanced because of constant cocaine use. Because CART peptide seems to oppose some of the effects of cocaine, the stimulatory effect of cocaine on CART gene expression suggests the existence of an endogenous feedback mechanism to attenuate the behavioral effects of cocaine. Therefore, the pharmacological evidence suggesting a role for CART peptide in the rewarding and reinforcing effects of psychostimulants is consistent with both anatomical and behavioral studies.

Behavioral Evidence Supporting a Role for CART in Psychostimulant Addiction

Central administration of CART peptide into the rat VTA mimics the stimulatory effects of psychostimulants by causing an increase in locomotor activity (LMA) and conditioned place preference (Kimmel et al., 2000). The CART-induced increase in activity was attenuated in a dose-dependent manner by the DA receptor antagonist haloperidol, supporting a dopaminergic-mediated mechanism. In contrast, injection of CART peptide into the accumbens had no effect on LMA; however, intra-accumbal CART administration attenuated LMA produced by systemically administered cocaine and amphetamine (Jaworski et al., 2003). Consistent with its effects on cocaine-induced LMA, CART peptide dose-dependently attenuated locomotion produced by intra-accumbal infusions of DA, thus indicating a direct modulation of mesolimbic DA (Jaworski et al., 2003). Because of CART peptide's dual role in the VTA (stimulatory) and NAc (attenuation of cocaine- and DA-induced behaviors), it is plausible that CART peptide modulates responses to reward and reinforcement. This hypothesis is supported by a recent study demonstrating that, after behavioral conditioning, exposure to cues associated with cocaine increased CART-immunoreactive neurons in the NAc (Mattson and Morrell, 2005), suggesting that, not only does cocaine itself affect CART levels, the conditioning and environmental cues associated with cocaine also increase CART peptide levels. This study suggests that the CART may mediate the cognitive effects of drug addiction and play an important role in long-term conditioning of drug-related cues.

CART knockout (KO) mice have been generated and used in studies examining the behavioral effects of psychostimulants. Unfortunately, the results have been contradictory. For instance, a decreased response to amphetamine and cocaine was observed in CART KO mice versus wild-type mice (Couceyro et al., 2005). Despite an increased ambulatory effect in response to high doses of amphetamine, the data suggest attenuation of psychostimulant-induced LMA in CART KO mice. In addition, unpublished studies from our laboratory demonstrated that CART KO mice are less sensitive to the reinforcing effects of cocaine because cocaine-induced LMA was blunted in CART KO mice. In contrast to these studies suggesting differences in drug-influenced behavior between wild-type and KO mice, a recent report (Steiner et al., 2006) indicated that there were no significant differences in cocaine-induced LMA or cocaine self-administration. The authors suggest that, although CART may serve a modulatory role in the effects of psychostimulants in intact animals, it is not essential if lacking throughout development. Reasons underlying the discrepancy in results are unclear; however, differences in both the strain of mouse and the way in which the KO mice were generated should be considered as possible explanations. Although the data and subsequent interpretations of the relationship between psychostimulants and the CART system may differ, it is apparent that CART modulates the reinforcing behavioral effects of psychostimulants.

The Role of CART Peptide in the Effects of Nonpsychostimulant Drugs of Abuse

CART also seems to be involved in modulating the actions of nonstimulant drugs of abuse. For instance, alcoholism in a

Korean population is associated with a mutation in the CART gene (Jung et al., 2004). DA receptor-sensitive increases in CART mRNA and peptide in the rat NAc was noted following ethanol administration (Salinas et al., 2006). CART peptides also act on μ-opioid receptors (Rothman et al., 2003) and enhance the spinal analgesic actions of morphine (Damaj et al., 2004). Finally, a cannabinoid CB1 antagonist, rimonabant, inhibited drug-induced food intake in wild-type but not CART-deficient mice (Osei-Hyiaman et al., 2005).

Conclusions

Despite some ambiguous reports, the majority of studies support the hypothesis that CART is an important regulator of appetite and psychostimulant addiction. Anatomically, CART peptides and/or mRNA are found in several brain regions associated with both feeding and reward/reinforcement, and there is a strong anatomical association between CART and other systems associated with feeding and the rewarding properties of psychostimulants. CART has been linked to leptin and glucocorticoids, two important regulators of feeding, as well as DA and GABA, which are involved in the rewarding effect produced by psychostimulants. In addition, several different lines of pharmacological and behavioral evidence suggest that CART peptides modulate the mesocorticolimbic dopaminergic system. Because of CART's interactions with mesolimbic dopamine system, although speculative, the CART system may have implications for other neurological disorders involving dopaminergic transmission. Indeed, obesity and drug abuse is highly prevalent among patients suffering from mental disorders such as schizophrenia. There is a great potential for CART as a therapeutic target for i) the treatment of human eating disorders, including obesity and anorexia nervosa, and ii) the treatment of psychostimulant addiction (Hunter and Kuhar, 2003). Despite advances in understanding CART's role in the central nervous and peripheral systems, much work is yet to be done in the CART field. One of the major challenges in the field has been the absence of an identifiable CART receptor; however, recent advances (Vicentic et al., 2006) could greatly facilitate understanding of pharmacological and behavioral effects of CART. These studies demonstrated for the first time signaling mechanisms and specific binding of CART peptide in a cell line supporting the existence of a specific CART receptor that is G-protein-coupled and functions via Gi/o mechanisms. These novel findings open a possibility for the future cloning of a CART receptor and the development of drugs that would target CART as a potential treatment of eating and drug abuse-related disorders.

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