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CART peptides increase 5-hydroxytryptamine in the dorsal raphe and nucleus accumbens of freely behaving rats

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Abstract

Cocaine and amphetamine-regulated transcript peptides (CART) are implicated in the antidepressant effect. This may involve in 5-hydroxytryptamine (5-HT) in the CNS. The aim of the present studies was to investigate the effect of CART peptides on extracellular 5-HT in the dorsal raphe nucleus (DRN) and nucleus accumbens (NAcc) using a microdialysis approach in freely-behaving rats. Reverse infusion of CART_{61–102} in the DRN produced a concentration (10 μ M–100 μ M) -dependent increase in 5-HT in the DRN. Similarly, CART_{62–76} (10 μ M–100 μ M) infused into the DRN and NAcc elevated 5-HT in the DRN and NAcc, respectively. Thus, CART increases extracellular 5-HT in both the DRN and NAcc. In addition, infusion of CART_{62–76} (100 μ M) in the DRN produced a significant increase in 5-HT in the NAcc, implying an existence of CART receptors responsible for the depolarization-dependent release. In summary, the results of the present studies suggest that CART peptides may have an antidepressant effect through increases in extracellular 5-HT.

Keywords

CART peptides; antidepressant; dorsal raphe; nucleus accumbens; microdialysis; serotonin

Cocaine- and amphetamine-regulated transcript peptides (CART) are a group of brain-derived substances induced by the administration of cocaine and amphetamine, and they are widely distributed throughout the brain including the dorsal raphe nucleus (DRN) and nucleus accumbens (NAcc) (Douglass et al. 1995; Hurd and Fagergren 2000; Koylu et al. 1998). The peptides are stored within the vesicles (Damaj et al. 2003), and thus may act as a neuromodulator (Smith et al. 1999). Physiologically, CART peptides produced hypophagia, hyperlocomotion and anxiety-like behavior (Chaki et al. 2003; Kimmel et al. 2000; Stanek 2006). At high doses, CART also induced analgesia and impaired locomotion (Damaj et al. 2003). While mounting evidence points to dopamine response (Philpot and Smith 2006; Vicentic and Jones 2007), some suggest an interaction between CART and the serotonergic

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system. For instances, 5-HT_{1A} receptor agonist buspirone attenuated the anxiogenic response to CART (Chaki et al. 2003). Increases in 5-hydroxytryptamine (5-HT; serotonin) turnover was implicated in the regulation of CART-induced hypophagia (Choi et al. 2003). Recently, it was found that patients with CART mutation had high depression scores, supporting a speculation that CART may have an antidepressant effect, probably through 5-HT in the brain (Miraglia del Giudice et al. 2006; Pae et al. 2006).

To understand the role of CART in depression, the aim of the present studies was to determine extracellular 5-HT in response to CART administration in freely-behaving rats. The experiments were primarily carried out in the DRN which contains the most serotonergic neurons projected to the forebrain, including the NAcc. CART_{61–102} (42 amino acid residues) and CART_{62–76} (15 amino acids) fragments were explored in this study since they are biologically active (Kimmel et al. 2002; Vaarmann and Kask 2001). The peptides (dissolved in the aCSF) were 10 μ M, 30 μ M and 100 μ M for reverse microdialysis infusion to the nuclei. Because of the probe membrane barrier, the actual concentration in the interstitial space would be considerably less than those infused. Nevertheless, the drug concentrations used in this study were believed to be within the acceptable physiological range.

Sprague-Dawley albino male rats obtained from Charles River Laboratories (Raleigh, NC, USA) were used in this investigation. All procedures of animal uses were in strict accordance with the *NIH Guide for the Care and Use of Laboratory Animals* and approved by the local Animal Uses Committee at Florida Atlantic University. All efforts were made to minimize the number of animals used and their suffering. Rats weighing 300–350g were anesthetized with a combination of xylazine (4 mg/kg, i.p.) and ketamine (80 mg/kg, i.p.). Guide cannulae (10 mm in length of a 22-gauge stainless steel tubing) were implanted 2 mm below the skull surface at the coordinates relative to interaural zero: +AP 1.2, ML 4.0 at an angle 32° lateral to midline for the DRN; and AP +10.7, ML 1.4 for the NAcc (Paxinos and Watson 1998). Experiments began no sooner than one week after surgery. The night before an experiment, the dialysis probes were inserted through the guide cannulae and secured with dental cement. The length of the steel shaft was adjusted to place a 1.0-mm-long segment of dialysis tubing in the DRN (DV 5.5–6.4, 32° angle) and 2.5-mm segment in the NAcc (DV 6.0–8.5). The probes were perfused with the artificial cerebrospinal fluid (aCSF) containing 140mM NaCl, 3.0 mM KCl, 1.5 mM CaCl₂, 1.0 mM MgCl₂, 0.25 mM NaH₂PO₄, and 1.0 mM Na₂HPO₄. The aCSF was pumped at a rate of 1.0 μ l/min. Samples were collected at 20 min intervals and analyzed by high-performance liquid chromatography with electrochemical detection (HPLC-EC; EiCOM HTEC-500) in conjugation with an autoinjector (CMA 200). Mobile phase (0.1 M phosphate buffer at pH 6.0, 500 mg/L 1-decanesulfonic acid, 50 mg/L EDTA, and 1.0% methanol) was pumped at a rate of 0.50 ml/min. The detection limit was 0.05 pg for 5-HT. Figure 1 shows examples of chromatography from the DRN before (panel A) and after drug infusion (panel B). In this study, mean basal 5-HT calculated as the average of the four successive samples before drug administration was 0.51 $\pm$ 0.07 pg/sample in the DRN (n=38) and 0.33 $\pm$ 0.04 pg/sample (n=24) in the NAcc. The data presented in figures are normalized to mean ($\pm$ s.e.m.) percent changes from the averaged baseline measurements. Unless otherwise noted, the statistical analysis was

performed with the percent changes using one-way repeated measures ANOVA, and if differences were found ($P < 0.05$), further tests were carried out to analyze individual time points compared with the vehicle control groups using Scheffe's *post-hoc* test. The significance was set at 0.05.

The DRN was examined first by CART_{61–102} infusion. As shown in figure 2, CART_{61–102} produced an increase in 5-HT in a dose-dependent manner ($F_{(3,18)} = 3.204$, $P = 0.048$). With respect to vehicle control, the infusion of 10 μM produced no consequential results ($F_{(1,8)} = 3.872$, $P = 0.0847$). However, a significant increase was found in response to 30 μM ($F_{(1,9)} = 5.449$, $P = 0.0437$) and 100 μM ($F_{(1,11)} = 6.843$, $P = 0.024$). The maximum effect was a 125.9% (s.e.m; $\pm 46.5\%$) rise in response to 30 μM . Over time, there was no additional increase as the dose approached 100 μM .

Thus, the results of our data are consistent with the general assumption that drugs that elevate extracellular 5-HT may have potential therapeutic uses in the treatment of depression. Antidepressants such as the 5-HT reuptake blockers and monoamine oxidase inhibitors are known to increase extracellular 5-HT in all brain areas including the DRN (Adell and Artigas 1991; Rutter and Auerbach 1993). Likewise, CART_{62–76} raised 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-HT, in the frontal cortex and hypothalamus (Vaarmann and Kask 2001). However, it appears that this peptide fragment had no effect in the hippocampus, but decreased 5-HIAA and 5-HT in the striatum. It should be kept in mind that the 5-HT turnover in that study was determined using a postmortem brain with homogenates containing components from both cytoplasmic synthesis and extracellular release. In the present study, only extracellular 5-HT, which is highly linked with antidepressant therapy, was determined. To understand the effect on extracellular 5-HT, CART_{62–76} peptide fragment was evaluated by infusing into the DRN and NAcc. As shown in figure 3, the CART_{62–76} peptide fragment evoked a concentration-dependent increase in the DRN ($F_{(3,18)} = 5.5$, $P = 0.0073$) and the NAcc ($F_{(3,14)} = 9.519$, $P = 0.0011$). The maximum increase above the baseline in the DRN and NAcc was 145.5% ($\pm 70.3\%$) and 117.2% ($\pm 35.4\%$), respectively. Upon comparison of CART_{62–76} and CART_{61–102} fragments, however, no difference in the increased 5-HT was found in the DRN.

The pharmacological profile that CART-induced increases in 5-HT were in a concentration-dependent manner implies a neuron-associated activity of neurotransmission. This effect could result from either depolarization-dependent or independent mechanisms. The depolarization-dependent mechanism is largely attributed to a direct CART stimulation on specific receptors, the effect blocked by selective antagonists. However, since little is known about the CART receptors (Vicentic et al. 2006) and the antagonists for CART peptides are not available, it is not possible to clarify these questions using a conventional single probe microdialysis. On the other hand, depolarization-independent occurrences that may also involve a CART-induced effect on 5-HT should not be dismissed. For example, 5-HT is elevated by activation of biosynthesis (i.e., tryptophan hydroxylase) or through suppression of the neurotransmitter clearance pathways (i.e., monoamine oxidase, reuptake transporter) (Arai et al. 2002; Fuller 1994). If this was the mechanism for CART-induced increases in 5-HT, serotonergic neurons in the DRN would not be depolarized. Instead, the neurons would be hyperpolarized due to activation of 5-HT_{1A} receptors following the increased 5-HT (Tao

et al. 2000). As a result, spontaneous 5-HT efflux would be subsequently decreased, especially in the axon terminal regions derived from DRN. Thus the mechanisms for mediating CART-induced increases in 5-HT in the DRN could be determined simply by measuring 5-HT response in the NAcc because serotonergic projections to this area are almost exclusively from the DRN (Matsuzaki et al. 1993; Van Bockstaele et al. 1993). Based on this hypothesis, 5-HT in the NAcc responding to DRN infusion could be: 1) decreased, suggesting that serotonergic neurons in the DRN were hyperpolarized due to activation of 5-HT_{1A} autoceptors; if this was the result, increases in 5-HT evoked by CART were probably due to activation of biosynthesis or inhibition of metabolic pathways; 2) increased, suggesting that the serotonergic neurons in the DRN were depolarized by CART infusion; or 3) unaltered, suggesting a net balance between hyperpolarized and depolarized effects. In the present study, a dual-probe microdialysis was carried out to examine the 5-HT response in the NAcc while CART peptides were reversely infused to the DRN. As shown in figure 4, 5-HT levels significantly increased in response to infusion of 100 μ M of CART₆₂₋₇₆ in the DRN ($F_{(1,10)}=18.837$, $P=0.0015$). The maximum increase was 45.0% ($\pm 6.1\%$). The effect is unlikely due to either excessive drug diffusion, considering the long distance between the two probes/nuclei, or drug doses, which were not much higher than those that are commonly used for microdialysis (Murphy et al. 1996; Yang et al. 2004). The results that significant increases were found in the NAcc certainly exclude a possible involvement of depolarization-independent mechanisms such as biosynthesis or metabolic pathways. A more reasonable interpretation is a depolarization-dependent mechanism, for instance, an existence of CART receptors in response to CART infusion. It is plausible that activation of CART receptors might increase in serotonergic neuronal discharge and thereby result in releases of 5-HT in the NAcc. This is consistent with neuroanatomical evidence that serotonergic innervations in the NAcc are almost exclusively from the DRN (Matsuzaki et al. 1993; Van Bockstaele et al. 1993). Likewise, the results of our data also suggest that the CART peptides had a direct excitatory effect on 5-HT axon terminals in the NAcc (figure 3B).

However, compared to the single probe experiments, increases in 5-HT in the NAcc were relatively small using the dual-probe microdialysis. Difference may be due to the size of axonal population influenced by CART peptides. It is known that 5-HT pool in the NAcc microdialysis is derived from many sub-areas of the DRN because of widely dispersal axonal projections (Van Bockstaele et al., 1993). However, CART in the DRN had the effect only on neurons surrounding the probe but not those outside the effective ranges of perfusion. Thus, unlike in the single probe microdialysis, only were a small fraction of axons in the NAcc influenced by CART infusion in the DRN. Nevertheless, these results support the conclusion that CART produced a depolarization-dependent increase in 5-HT in both the DRN and NAcc.

In conclusion, the results of the present study demonstrate that CART peptides induce 5-HT release in both the DRN and NAcc. Moreover, 5-HT release in the NAcc can be increased both by stimulating CART receptors in the NAcc and by depolarizing serotonergic neurons in the DRN. Conceivably, increasing 5-HT after CART peptides would activate 5-HT receptors. To date, at least fourteen subtypes of 5-HT receptors have been identified, including 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C} and 5-HT₃ receptors in the NAcc (Bruinvels et al. 1993;

Dremencov et al. 2006; Ma et al. 2006). Neurologically, NAcc, which belongs to dopaminergic mesolimbic and mesocortical systems, is associated with hedonic and motivational actions such as drug rewarding and reinforcement. However, the NAcc is emerged to play a role in depressive behavior because of the intimated interaction between dopaminergic systems and 5-HT receptors found in this region (Elhwuegi 2004). Activation of 5-HT receptors may lead to an increase in dopamine (Boulenguez et al. 1998; Yan and Yan 2001) and possibly potentiate a therapeutic effect of antidepressants (Dremencov et al. 2006). It is very possible that the effect can be enhanced after activation of serotonergic neurons in the DRN. Thus, a better understanding of the neural circuitry connecting the DRN to the NAcc should lead to further appreciation of drug treatment and the mechanism of CART-mediated behavioral consequences. In conclusion, the results of our data suggest that CART induces an increase in extracellular 5-HT, supporting the hypothesis that CART peptides may have an antidepressant effect (Miraglia del Giudice et al. 2006; Pae et al. 2006).

Acknowledgments

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Figure 1.

Chromatograms obtained from 10 μ l microdialysis samples collected in the DRN under baseline conditions (A) and CART₆₁₋₁₀₂ infusion (30 μ M; B). The 5-HT peaks were elevated from 0.44 pg (A) to 0.98 pg (B).

Figure 2.

Extracellular 5-HT levels in the DRN in response to infusion of CART₆₁₋₁₀₂. Reverse microdialysis infusion of CART₆₁₋₁₀₂ produced concentration-dependent increases in 5-HT in the DRN. All data are mean $\pm$ SEM. * p <0.05 significantly different between 100 μ M and vehicle and # p <0.05 between 30 μ M and vehicle.

Figure 3.

Effects of CART₆₂₋₇₆ on extracellular 5-HT in the DRN (A) and NAcc (B). CART₆₂₋₇₆ infused into the DRN and NAcc induced increases in 5-HT in the DRN and NAcc, respectively. All data are mean $\pm$ SEM. * p <0.05 significantly different between 100 μ M and vehicle and # p <0.05 between 30 μ M and vehicle.

Figure 4.

Dual-probe microdialysis of extracellular 5-HT in the NAcc. Reverse microdialysis infusion of CART₆₂₋₇₆ in the DRN evoked an increase in 5-HT levels in the NAcc. All data are mean $\pm$ SEM. Significant difference (* $p < 0.05$) between CART and vehicle