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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 (10–100 μM) -dependent increase in 5-HT in the DRN. Similarly, $CART_{62-76}$ (10–100 μM) 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 μM) 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) [8,12,15]. The peptides are stored within the vesicles [7], and thus may act as a neuromodulator [24]. Physiologically, CART peptides produced hypophagia, hyperlocomotion and anxiety-like behavior [5,13,25]. At high doses, CART also induced analgesia and impaired locomotion [7]. While mounting evidence points to dopamine response [22,29], some suggest an interaction between CART and the serotonergic system. For instances, 5-HT_{1A} receptor agonist buspirone attenuated the anxiogenic response to CART [5]. Increases in 5-hydroxytryptamine (5-HT; serotonin) turnover was implicated in the regulation of CART-induced hypophagia [6]. 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 [18,20].

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 [14,27]. The peptides (dissolved in the aCSF) were 10, 30 and 100 μM 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 300–350 g were anesthetized with a combination of xylazine

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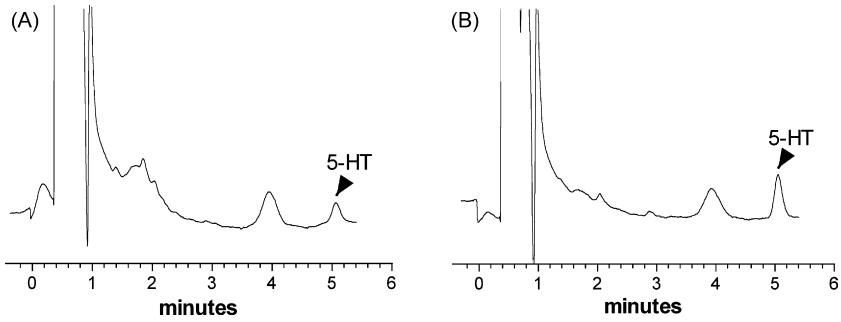

Fig. 1. Chromatograms obtained from 10 μl microdialysis samples collected in the DRN under baseline conditions (A) and $CART_{61–102}$ infusion (30 μM; B). The 5-HT peaks were elevated from 0.44 pg (**A**) to 0.98 pg (**B**).

(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 [21]. Experiments began no sooner than 1 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 6.0.0–8.5). The probes were perfused with the artificial cerebrospinal fluid (aCSF) containing 140 mM NaCl, 3.0 mM KCl, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 0.25 mM NaH_2PO_4, and 1.0 mM Na_2HPO_4. The aCSF was pumped at a rate of 1.0 μl/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. Fig. 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 ± 0.07 pg/sample in the DRN ($n = 38$) and 0.33 ± 0.04 pg/sample ($n = 24$) in the NAcc. The data presented in figures are normalized to mean (±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 Fig. 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; ±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 [1,23]. Likewise, $CART_{62–76}$ raised 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-HT, in the frontal cortex and hypothalamus [27]. 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 Fig. 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

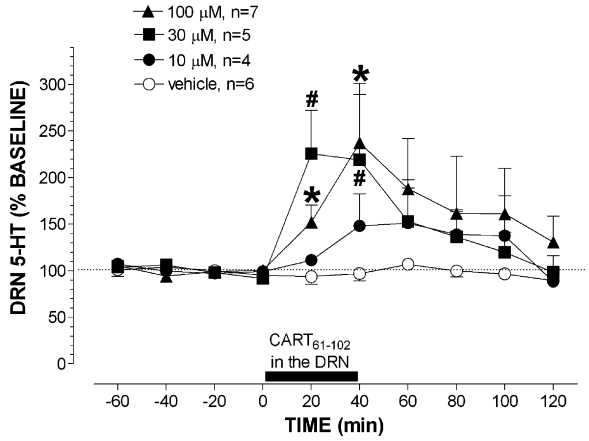

Fig. 2. Extracellular 5-HT levels in the DRN in response to infusion of $CART_{61–102}$. Reverse microdialysis infusion of $CART_{61–102}$ produced concentration-dependent increases in 5-HT in the DRN. All data are mean ± S.E.M. $^{*}P < 0.05$ significantly different between 100 μM and vehicle and $^{\#}P < 0.05$ between 30 μM and vehicle.