

Research Article

Intraperitoneal Administration of CART 55-102 Inhibits Psychostimulant-Induced Locomotion

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Abstract CART (cocaine and amphetamine regulated transcript) peptide functions as both a neurotransmitter and a hormone and is found both in the central nervous system (CNS) and in the periphery. CART peptide in the nucleus accumbens (NAc) has been implicated in the regulation of cocaine-dopamine-mediated locomotion and self-administration, and amphetamine-mediated locomotion and behavior. However, there are no studies on the effect of systemic administration of CART peptide on cocaine and amphetamine-mediated locomotion. In this study, we tested if the systemic administration of CART 55-102 by the intraperitoneal (ip) route has a functional effect on psychostimulant-mediated locomotion in rats as it does when given into the brain. We determined that ip CART 55-102 attenuates psychostimulant-mediated locomotion as it does when administered into the NAc and display a biphasic dose response curve.

Keywords CART peptide; cocaine; amphetamine; locomotion

1 Introduction

CART (cocaine and amphetamine regulated transcript) peptide functions as both a neurotransmitter and a hormone and is found both in the central nervous system (CNS) and in the periphery [28]. Much of the current research on CART peptide and psychostimulant addiction has focused on the activity of CART 55-102 in the nucleus accumbens (NAc) because that nucleus is involved in addiction and the CART expression in this region of the brain is affected by psychostimulants [5,8]. CART in the NAc has been implicated in the regulation of cocaine-dopamine-mediated locomotion and self-administration [13,15] and amphetamine-mediated locomotion and behavior [17,18]. However, CART 55-102 also acts at extra-accumbal sites including the ventral pallidum, the ventral tegmental area (VTA), the basolateral amygdala and the paraventricular thalamus to regulate psychostimulant-mediated behavior [9, 12,14,27], suggesting that the effects of CART peptide on psychostimulant-mediated behavior can be more

widespread, in keeping with the widespread distribution of the peptide [20,21].

While it might be assumed that a 48 amino acid peptide like CART 55-102 would not cross the blood brain barrier (BBB) to any significant extent, there is some evidence that, in fact, CART peptide does cross the BBB by a non-saturable, simple diffusion mechanism [16]. Kastin and Akerstrom [16] showed that 58% of injected CART 55-102 (intravenous, iv) reached the brain rapidly and in intact form. Furthermore, other studies showed that the effects of centrally administered CART peptide (icv) and intravenously administered CART peptide (iv) on neuroendocrine state were similar [1,2,4,29,30, 34]. Interestingly, intraperitoneal injection of the single chain variable fragment of a CART antibody attenuated the expression of cocaine-induced sensitization in mice, suggesting a connection between peripheral CART peptides and the central actions of psychostimulants [3]. While these studies suggest that CART 55-102 crosses into the brain when administered systemically to exert physiological effects [1,2,4,29,30,34], other studies do not support this idea. For example, icv CART 55-102 elicited dose-related increases in mean arterial pressure and renal sympathetic nerve activity whereas iv injection of the same dosage of CART 55-102 (1 nmol) as that used in the icv experiment failed to cause any cardiovascular and renal sympathetic nerve responses [26]. Also, intracisternally (i.c.) administered CART peptide fragments (CART 61-102 and CART 55-102) dose-dependently (1–4 nmol) increased heart rate and blood pressure in urethane-anesthetized adult male Sprague-Dawley rats whereas iv administrations of these peptides showed little or no effects on the heart rate and blood pressure in the rat [10]. Therefore, it will be interesting to determine if ip CART 55-102 exerts any effects on psychostimulant-mediated behavior. In this study, we tested if the systemic administration of CART 55-102 by the intraperitoneal (ip) route had a functional effect on psychostimulant-mediated locomotion in rats as it does when given into the brain.

2 Methods

2.1 Animals

Male Sprague Dawley rats, weighing between 225 and 275 g at the time of purchase (Charles River Inc., Wilmington, MA, USA), were used for the behavioral studies. The rats were pair-housed and were provided rat chow and water ad libitum, and maintained on a 12-hour light: dark cycle (lights on at 7 am). Experiments and animal care were in accordance with the Institute of Animal Care and Use Committee of Emory University and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

The rats were 410–610 g at the time of experiments. Rats were habituated to handling, ip injections, and the locomotor chambers before the actual experiments. On an experimental day, locomotor testing took place between 12 noon and 7 pm. The testing days were at least 72 hours apart. Rats were randomly assigned to treatment groups. Rats were used 1–5 times for Locomotor Activity (LMA) studies throughout the course of all the experiments. A total of 40 rats were used for this study.

2.2 Drugs

CART 55-102 (American Peptide Company, Sunnyvale, CA, USA), CART 1-27 (Emory Microchemical Facility, Emory University, Atlanta, GA, USA), D-Amphetamine hemisulfate (Sigma-Aldrich, St. Louis, MO, USA), and cocaine hydrochloride (NIDA) were used in these experiments. All drugs were dissolved in saline for intraperitoneal injection. Drugs were injected intraperitoneally in a volume of 1 mL/kg. The drug injections had no adverse effects on the rats.

2.3 Locomotor activity (LMA)

LMA was measured in 5-minute intervals using a photocell cage (Omnitech Electronics, Columbus, OH, USA) with the dimensions of 40 × 40 × 30 cm. The cages were made of transparent plexiglass walls and contained 32 photobeams located 5 cm above the floor to record LMA. Each cage was housed in a stainless steel box and connected to a computer equipped with software (Digipro; Omnitech Electronics) to measure LMA. On testing days, animals were habituated to the chambers for 30 minutes, followed by 30 minutes of basal LMA measurement. After basal LMA data were taken, animals were taken out of the chambers and given an ip injection of saline or CART peptides and returned to the chamber for 15 minutes of LMA measurement. Afterwards, rats were taken out and given an ip injection of saline, cocaine or amphetamine, depending on the experiment. LMA was then measured for an additional 30 or 60 min as indicated in the text.

2.4 Statistics

Data are all expressed as mean ± SEM, and significance occurs when $P < .05$ (** $P < .05$, ** $P < .01$, *** $P < .001$).

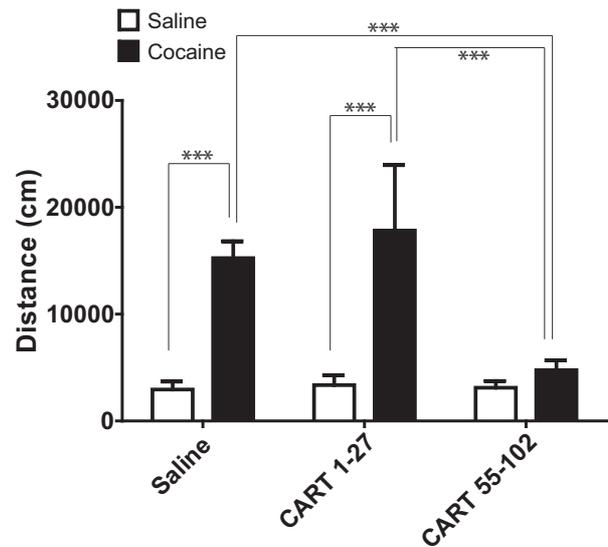


Figure 1: CART 55-102 attenuates cocaine-mediated locomotion. Saline ($n = 12, 20$), CART 1-27 ($14.5 \mu\text{g}/\text{kg}$ ip) ($n = 6, 7$), and CART 55-102 ($25 \mu\text{g}/\text{kg}$ ip) ($n = 4, 11$) were administered to rats 15 minutes before saline or cocaine ($10 \text{ mg}/\text{kg}$ ip, $1 \text{ mL}/\text{kg}$ volume) challenge, and locomotor activity (LMA) was measured for 60 minutes. The notation above ($n = a, b$) is such that $a = n$ for saline and $b = n$ for cocaine treatment groups. The doses of CART 1-27 ($14.5 \mu\text{g}/\text{kg}$ ip) and CART ($25 \mu\text{g}/\text{kg}$ ip) are equimolar. The y-axis represents distance traveled in 60 minutes. A 2-way ANOVA shows that there is a significant effect of cocaine ($F_{1, 54} = 24.61, P < .0001$), treatment ($F_{2, 54} = 3.704, P = .0311$), and a cocaine × treatment interaction ($F_{2, 54} = 3.642, P = .0328$). The differences determined by Bonferroni *post hoc* tests are shown in the figure: *** $P < .0001$.

All analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). LMA data were analyzed as total locomotor activity—a summation of individual locomotor activity values post-saline, post-cocaine or post-amphetamine injection. For analysis of the total locomotor activity data, a 2-way ANOVA with Bonferroni post test was used. In all analyses, distance (total locomotor activity) was the dependent variable. For general comparative systemic effect studies (Figures 1 and 2), the independent variables were CART (3 levels—saline, CART 1-27, CART 55-102), cocaine (2 levels—0, $10 \text{ mg}/\text{kg}$), and amphetamine 2 levels—0, $2 \text{ mg}/\text{kg}$), depending on the experiment. In these analyses, a 2-way ANOVA was used to check if there were main effects of the independent variables CART, cocaine, and amphetamine and also to determine if there was a CART × cocaine and CART × amphetamine interaction, where applicable.

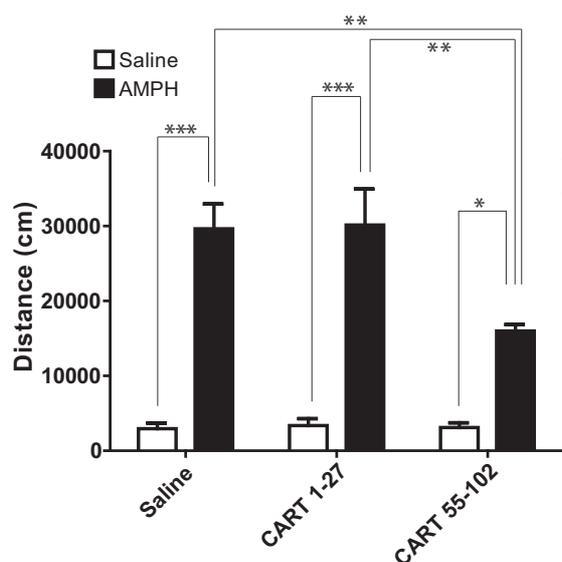


Figure 2: CART 55-102 attenuates amphetamine-mediated locomotion. Saline ($n = 8, 12$), CART 1-27 ($14.5 \mu\text{g}/\text{kg}$ ip) ($n = 6, 7$), and CART ($25 \mu\text{g}/\text{kg}$ ip) ($n = 4, 5$) were administered to rats 15 minutes before saline or amphetamine ($2 \text{ mg}/\text{kg}$ ip, $1 \text{ mL}/\text{kg}$ volume) challenge and LMA was measured for 60 minutes. The notation above ($n = a, b$) is such that $a = n$ for saline and $b = n$ for amphetamine treatment groups. A 2-way ANOVA shows that there is a significant effect of amphetamine ($F_{1, 36} = 114.2, P < .0001$), treatment ($F_{2, 36} = 4.245, P = .0221$), and an amphetamine \times treatment interaction ($F_{2, 36} = 4.225, P = .0225$). The differences determined by Bonferroni *post hoc* tests are shown in the figure: $*P < .05$, $**P < .01$, $***P < .001$.

For systemic dose response effect studies (Figures 3 and 4), the independent variables were CART 55-102 dose (4–5 levels, depending on the experiment), cocaine (2 levels—0, 10 mg/kg), and amphetamine (2 levels—0, 2 mg/kg), depending on the experiment. In these analyses, a 2-way ANOVA was used to check if there were main effects of the independent variables CART 55-102 dose, cocaine, and amphetamine and to determine if there was a CART 55-102 dose \times cocaine and CART 55-102 dose \times amphetamine interaction, where applicable. If 2-way ANOVA revealed an effect, it was followed up with Bonferroni *post hoc* tests to determine where the differences lie and the differences are shown in the figures as $*P < .05$, $**P < .01$, $***P < .001$.

3 Results

Animals were treated as described in Section 2. Administration of cocaine (ip, 10 mg/kg) increased LMA in all cases compared to injection of saline. Injection of CART 1-27, an inactive peptide control, given at the same equimolar dose as CART 55-102, had no effect on cocaine-induced LMA,

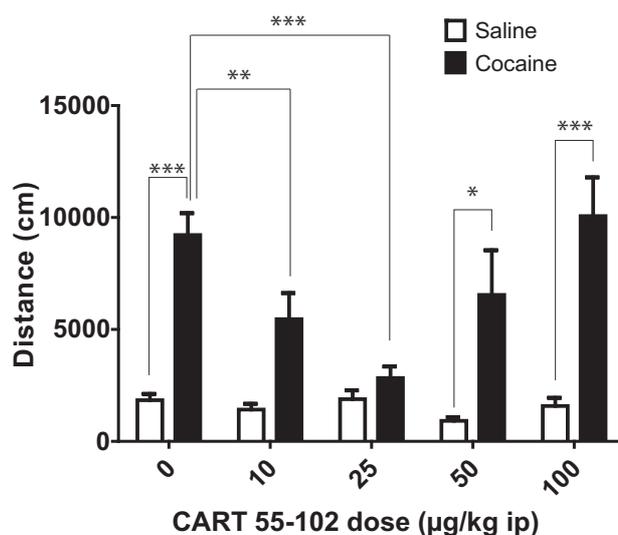


Figure 3: CART 55-102 exhibits a U-shaped dose-effect curve on cocaine-mediated locomotion. CART 55-102 in several doses ($0 \mu\text{g}/\text{kg}$ ip, $n = 20, 20$; $10 \mu\text{g}/\text{kg}$ ip, $n = 4, 8$; $25 \mu\text{g}/\text{kg}$ ip, $n = 11, 13$; $50 \mu\text{g}/\text{kg}$ ip, $n = 3, 4$; and $100 \mu\text{g}/\text{kg}$ ip, $n = 3, 5$) was administered to rats 15 minutes before saline or cocaine ($10 \text{ mg}/\text{kg}$ ip, $1 \text{ mL}/\text{kg}$ volume) challenge and LMA was measured for 30 minutes. The notation above ($n = a, b$) is such that $a = n$ for saline and $b = n$ for cocaine treatment groups. A 2-way ANOVA of distance shows that there is a significant effect of cocaine ($F_{1, 81} = 51.49, P < .0001$), CART 55-102 dose ($F_{4, 81} = 5.741, P < .0001$), and a cocaine \times CART dose interaction ($F_{4, 81} = 5.782, P < .0001$). The differences determined by Bonferroni *post hoc* tests are shown in the figure: $*P < .05$, $**P < .01$, $***P < .001$.

but injection of CART 55-102 reduced the LMA induced by cocaine (Figure 1). Furthermore, injection of CART 1-27 (equivalent molar dose as $25 \mu\text{g}/\text{kg}$ ip CART 55-102) had no effect on amphetamine-induced LMA, but injection of CART 55-102 reduced the LMA induced by amphetamine (Figure 2). In dose-response studies, a striking U-shaped dose-response curve was obtained. The dose of $25 \mu\text{g}/\text{kg}$ CART 55-102 produced the greatest reduction in cocaine-induced-LMA (Figure 3) and amphetamine-induced LMA (Figure 4). CART 55-102 had greater inhibitory effects (near 100%) on cocaine-induced LMA compared to amphetamine-induced LMA (about 50%) (Figures 1–4). Administration of CART 55-102 alone did not affect basal LMA (not shown).

4 Discussion

This is the first study investigating the effect of systemic administration (ip) of CART 55-102 on psychostimulant-mediated LMA. It was found that CART 55-102 was effective in reducing cocaine- and amphetamine-mediated LMA.

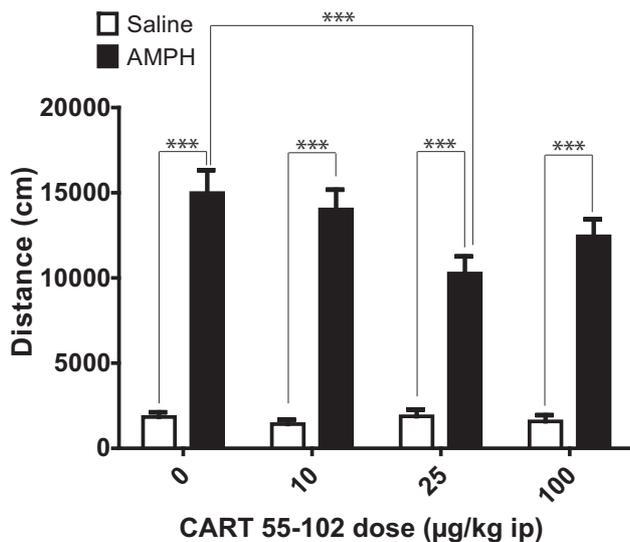


Figure 4: CART 55-102 exhibits a U-shaped dose-effect curve on amphetamine-mediated locomotion. CART 55-102 was given in several doses (0 µg/kg ip, $n = 8, 20$; 10 µg/kg ip, $n = 4, 4$; 25 µg/kg ip, $n = 5, 13$; 100 µg/kg ip, $n = 3, 4$) was administered to rats 15 minutes before saline or amphetamine (2 mg/kg ip, 1 mL/kg volume) challenge and LMA was measured for 30 minutes. The notation above ($n = a, b$) is such that $a = n$ for saline and $b = n$ for amphetamine treatment groups. A 2-way ANOVA of distance shows that there is a significant effect of amphetamine ($F_{1, 53} = 341.9, P < .0001$), CART 55-102 dose ($F_{3, 53} = 4.372, P = .0080$), and an amphetamine \times CART dose interaction ($F_{3, 53} = 4.644, P = .0059$). The differences determined by Bonferroni post hoc tests are shown in the figure: *** $P < .001$.

The data strongly support the idea that CART 55-102, when administered peripherally, crosses the blood brain barrier and exerts physiological effects [16]. The inhibitory effect of CART 55-102 after systemic administration is similar to the inhibitory effect seen when CART 55-102 is administered directly into the NAc [15,17]. However, in this study, we cannot be sure that the initial site of action of CART 55-102 is the brain: a peripheral site may be involved and may somehow promote the behavioral effect. In many studies, inactive CART 1-27 is used as a peptide control for CART 55-102 [15,23,37]. One limitation of this study is that we do not know if CART 1-27 ip crosses the BBB to the same extent as CART 55-102 ip. However, we do know that CART 1-27 is inactive because our data showed that it (like saline ip) does not alter psychostimulant-mediated LMA.

It was also found that systemic administration of CART 55-102 exhibited a U-shaped dose-response curve with a maximum inhibition found at 25 µg/kg ip. Higher or

lower doses had less of an inhibitory effect. This result agrees with Dylag et al. [7] who determined that the CART fragment (CART 85-102), when administered icv, attenuates cocaine- and amphetamine-mediated locomotion in mice in a biphasic manner—the CART fragment was effective at icv dose of 0.1 µg but not at a higher dose [6,7]. Our study suggests that CART 55-102 is generally inhibitory on psychostimulant-induced effects. This is compatible with the observation that CART 55-102 inhibitory effects on psychostimulant-mediated behavior have been found in many other brain sites, including the basolateral amygdala, ventral pallidum, VTA, and paraventricular thalamus [9, 12,14,27], in addition to the NAc [5,13,15,18]. While rewarding and locomotor-activating effects of CART 55-102 have also been observed, they have been under different conditions [19,36].

There may be many reasons for the U-shaped dose-response curve for CART 55-102 found in this study. It may suggest the presence of at least two CART peptide receptors—one a high affinity site involved in psychostimulant effects and the other a low affinity site not involved in psychostimulant effects or perhaps opposed to the effects of the high affinity site. For instance, intra-basolateral amygdala infusions of CART 55-102 (1 µg/side) produced neither conditioned place preference (CPP) nor conditioned place aversion (CPA), infusions of CART 55-102 (2 µg/side) produced CPP whereas a higher dose of 4 µg/side produced CPA [27]. Furthermore, it has been proposed that in either the VTA or the NAc, CART 55-102 may have some small psychostimulant-like effects, but then it tends to oppose the actions of psychostimulants at higher doses [22]. This idea of at least two CART sites may explain why CART 55-102 has been characterized as cocaine-like and anti-cocaine at the same time—intra-VTA administration of CART 55-102 produces locomotor activity [19] and yet attenuates cocaine-mediated locomotion [14]. While receptors for CART peptides have not yet been cloned, they have been identified by binding and signaling [24,25,28].

It is interesting to note that while 10 µg/kg ip of CART 55-102, which equates to 5 µg ip in a 500 gm rat, is effective in attenuating cocaine-mediated locomotion by about 75% (see Figure 3), a 2.5 µg dose administered directly into the NAc is only partially effective in attenuating cocaine-mediated locomotion [15]. This implies that CART is more potent in attenuating cocaine-mediated locomotion when administered systemically than when administered intra-NAc. This suggests that there are likely to be some other sites of action than the NAc. This idea is indirectly supported by evidence that shows that when CART is administered icv, it induces c-fos in many regions but not in the NAc [35]. It is also possible that when CART 55-102 is injected into the NAc, it is diffusing to another site to produce its actions.

CART peptides were found in the blood and exhibited a diurnal variation [31,32,33]. Endogenous CART peptides were reported to be in the blood at about 100 pg/mL [31] or about 29 pM. The injected dose of 25 $\mu\text{g}/\text{kg}$ used in this study represents a mole dose of 2.4 nmoles in about a 500 gm rat. If we assume that such a rat has a blood volume of about 30 mls, and all of the CART peptide enters the blood, then one would expect a concentration of CART peptide to be a maximum of 0.42 $\mu\text{g}/\text{mL}$, or 0.08 nmoles/mL or 0.08 μM or 80 nM. Thus, the injected dose of CART 55-102 that has an inhibitory effect on psychostimulant-mediated LMA is much greater than the endogenous levels. This suggests that the ability of CART peptides normally in the serum (endogenous) to inhibit psychostimulant effects is unlikely to be physiologically important because the serum never has such a high level. Nevertheless, we have shown that 10- and 25 $\mu\text{g}/\text{kg}$ ip CART 55-102 attenuate cocaine (10 mg/kg ip)-mediated locomotion, and that 25 $\mu\text{g}/\text{kg}$ ip CART 55-102 attenuates amphetamine (2 mg/kg ip)-mediated locomotion. These doses are similar to the doses used in other studies that have detected physiological effects of systemic CART 55-102 [2,4,11]. Since there were no adverse effects on the rats at these doses but there were pharmacological effects, it is likely that ip CART 55-102 may have utility in modulating psychostimulant effects although the mechanism involved in this effect is unknown at this time.

5 Conclusions

This study suggests that CART peptides may be injected directly into the body to modulate psychostimulant effects and this may have utility in clinical interventions.

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