Intracerebroventricular CART peptide reduces food intake and alters motor behavior at a hindbrain site

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Received 8 February 2001; accepted in final form 17 August 2001

Aja, Susan, Shirin Sahandy, Ellen E. Ladenheim, Gary J. Schwartz, and Timothy H. Moran. Intracerebroventricular CART peptide reduces food intake and alters motor behavior at a hindbrain site. Am J Physiol Regulatory Integrative Comp Physiol 281: R1862–R1867, 2001.—Peptides from cocaine- and amphetamine-regulated transcript (CART) reduce food intake in rats when injected into the lateral ventricle. Hypothalamic and hindbrain sites important in the control of feeding contain CART-immunoreactive fibers. To further define the site of CART’s anorectic action, we compared feeding and other behavioral responses to third or fourth ventricular (3V, 4V) CART-(55–102) in 6-h food-deprived rats, both before and after cerebral aqueduct occlusion. 3V CART reduced the volume of Ensure consumed and resulted in fewer observations of eating and grooming within the 30-min test session. These reductions were significantly attenuated by aqueduct obstruction. 4V CART suppressed Ensure intake and resulted in decreased observations of feeding both with and without aqueduct blockade. 3V CART produced flat-backed postures and movement-associated tremors that were prevented by aqueduct obstruction. 4V CART also produced these signs, both with and without aqueduct blockade. We conclude that the major hypophagic effect of intracerebroventricular CART is mediated at a hindbrain site. The association of CART-induced feeding suppression with altered motor behavior questions the specificity of intracerebroventricular CART for actions on feeding.

CART is expressed in hypothalamic regions thought to be important in the control of food intake, including the dorsomedial, ventromedial, lateral, paraventricular, and arcuate nuclei. Some evidence suggests that arcuate CART may mediate some of the anorexigenic actions of leptin. The fall in leptin with fasting results in a decrease in CART mRNA (1), and CART peptide levels are decreased in leptin-deficient ob/ob mice and increased with leptin treatment in starved rats (10, 21, 28, 29). CART mRNA and peptide are also found in hindbrain sites involved in the control of feeding, including the nucleus of the solitary tract, locus ceruleus (8), parabrachial nucleus, and raphe nuclei (19).

Recombinant CART peptide from the short form of rat CART mRNA [rsCART-(42–89); nomenclature in Ref. 22] (21, 30) and other CART fragments (26) potently inhibit intake of solid food when injected into the lateral ventricle. rsCART-(42–89) given intracerebroventricularly also induces c-Fos production in structures of the rat brain that are involved in feeding behavior, including the hypothalamus, amygdala, parabrachial nucleus, and the nucleus of the solitary tract (30). Evidence supporting an anorectic effect of endogenous CART comes from the observation that antisera raised against CART peptides stimulate feeding when given intracerebroventricularly (21, 26). However, it is not yet clear that intracerebroventricular CART peptides have selective effects on food intake.

rsCART-(42–89) has been noted to produce movement-associated tremors when given intracerebroventricularly (21, 26). However, we have recently shown that rlCART-(55–102), a synthetic CART peptide fragment indicated by the long form of rat mRNA, but identical in amino acid sequence to rsCART-(42–89), produces these tremors as well (2). rlCART-(55–102) reduces intake of a nutritionally complete liquid diet when given via the lateral ventricle, but it alters licking patterns in a manner to suggest that CART has a significant slowing effect on oral motor function, rather than an enhancing effect on satiety per se (2). Thus possibilities remain that intracerebroventricular CART’s reported anorexigenic effect is secondary to altered or impaired motoric function or that a selectively hypophagic action is simply obscured by these other behavioral changes.

CART peptides injected into the lateral ventricle would reach hindbrain sites surrounding the fourth ventricle (4V) as well as hypothalamic sites near the third ventricle (3V). A number of peptides that affect
food intake have been demonstrated to have caudal hindbrain sites of action (5, 6, 14, 17, 18, 25). The present study was aimed at examining the possibility of a caudal hindbrain site of action for CART. As well as measuring feeding responses, we also examined the effects of CART on other behaviors. Because lateral ventricular CART has been noted to alter motor behavior (21, 30), another goal was to determine whether CART’s feeding and motor effects could be dissociated by a different ventricular site of administration.

METHODS

Male Sprague-Dawley rats (275–300 g) obtained from Charles River (Kingston, NY) were individually housed in hanging wire cages on a 12:12-h light-dark cycle and handled daily. We identified animals that avidly ingested vanilla-flavored Ensure (1.05 kcal/ml; Ross Laboratories, Columbus, OH) from graduated drinking bottles with stainless steel drinking tubes.

Rats were stereotaxically implanted with stainless steel cannulas aimed at either the 3V or 4V (3V, n = 33 total, 2 trials: 328 ± 2 g; 4V, n = 14 total: 282 ± 5 g at surgery). Rats were anesthetized with a 3:4 xylazine (20 mg/ml):ketamine HCl (100 mg/ml) cocktail administered intramuscularly (1 ml/kg) and placed in a stereotaxic instrument with the incisor bar adjusted to achieve a flat skull position. For 3V surgeries, a hole was drilled in the skull 1.0 mm caudal to bregma and 1.0 mm lateral to midline, and a 23-gauge stainless steel guide cannula was lowered 7.0 mm below dura, at a 10° angle toward midline. For 4V surgeries, a hole was drilled midline in the skull, 2.5 mm anterior to the occipital crest, and a 23-gauge cannula was lowered 5.7 mm below dura. Each 3V- and 4V-cannulated rat also had a 19-gauge stainless steel cannula aimed at the cerebral aqueduct. The aqueduct cannula was introduced into the brain through a hole drilled in the skull 8.0 mm caudal to bregma and 1.0 mm lateral to midline. The cannula was lowered 5.0 mm below dura at an 11° angle toward midline. Cannulas were secured in place using dental cement and stainless steel screws implanted in the skull. Thirty-gauge stainless steel obturators were inserted into the 3V and 4V cannulas and 22-gauge obturators were inserted into the aqueduct cannulas to maintain patency. Rats were given penicillin (300,000 U/ml; 0.2 ml im) to prevent postoperative infection.

After 1 wk of postoperative recovery, 3V cannula placements were assessed by examining water intake in response to intracerebroventricular ANG II. For this test, rats were deprived of water for 1 h, injected intracerebroventricularly with ANG II (50 ng/5 μl) or 0.9% sterile saline vehicle, and allowed 30-min access to water in graduated drinking tubes. Rats whose water intakes after ANG II were at least 5 ml greater than their intakes after saline injection were selected for experiments. 4V cannula placements were evaluated by measuring 30-min intakes of Ensure in response to bombesin (10 pmol) or saline vehicle (3 μl). Animals whose Ensure intakes were suppressed by at least 25% after bombesin were selected for experiments. All rats were adapted to a 6-h chow deprivation, followed by 30-min Ensure access, both during the light. Chow was returned to the rats for overnight access.

We used a synthetic CART peptide fragment indicated by the long form of rat CART mRNA [rCART-(55–102), CART] (American Peptide, Sunnyvale, CA). Rats were injected intracerebroventricularly with sterile 0.9% saline vehicle (3V: 3–5 μl; 4V: 3 μl) or 1 μg of CART in either the 3V or 4V just before Ensure access. We have previously shown that this dose of CART is threshold for reducing liquid diet intake in number of licks, altering specific parameters of licking microstructure, and changing gross motor behavior (2). Injections were made with a Gilmont microliter syringe attached to polyethylene tubing and a 30-gauge stainless steel injector, which extended 1.5 mm past the tip of the guide cannula. Effects of saline and CART in the 3V or 4V on food intake and other behaviors were tested on subsequent days. Three days after this set of CART injections, the cerebral aqueduct was plugged to block the rostrocaudal flow of cerebrospinal fluid from the 3V to the 4V. With the use of a method adapted from Ritter et al. (27), we used a 100-μl Hamilton syringe to inject 6 μl of lamblack-pigmented silicone grease (1:1 mixture of High Vacuum Grease (Dow Corning);Dielectric Connector Grease (Versachem)) into the cerebral aqueducts of lightly anesthetized rats. Thirty to 60 min later, rats were injected with saline in the 3V or 4V and tested during their regularly scheduled 30-min Ensure access. Behavioral responses to CART were measured on the following day.

Cumulative intakes of Ensure were measured every 3 min during the 30 min of liquid diet access. At each of the 10 time points, we observed each rat for 10 s and recorded whether or not rats exhibited behaviors that we had observed in earlier work (2). These behaviors were divided into two categories: prandial and postprandial behaviors normally exhibited by rats (eating, resting, grooming, and exploring) (3), and those noted by us (2) and others (21, 30) when rats are given intracerebroventricular CART (flat-backed and arched-backed postures and movement-associated tremors). We defined the prandial and postprandial behaviors as follows: eating was ingestion of liquid diet at the time of observation; resting was inactivity, whether standing, reclining, or sleeping; grooming was scratching or licking the body; and exploring was moving about the cage or rearing. Rats in the flat-backed body posture lay with their bellies flat against the bottom of the cage floor. Rats in the arched-backed posture had their backs arched and appeared to have generalized muscle tension. Movement-associated tremors involved tremors of the head and, in more severe cases, the entire body, but only when the animal was in motion. To verify this sign by a consistent method, we stimulated the animal at the end of each observation time point with a puff of air directed into the cage. It should be noted that it was possible for a rat to exhibit more than one type of behavior during any given 10-s observation period.

At the end of the experiments, rats were injected intracerebroventricularly (3V or 4V) with Evans blue ink and killed immediately for examination of ink and silicone plug locations. Data from animals with plugs in the cerebral aqueduct or aqueduct/rostral 4V, and with ink retained in the appropriate ventricle, were retained in the data set for statistical analysis. Of the original 33 3V and 14 4V rats, some did not complete the study because of apparent inaccurate cannula placement, decline of cannula patency, or inappropriate plug or ink locations at the end of the study. Thus 10 3V and 8 4V rats qualified for statistical analysis, which included repeated-measures ANOVA followed by planned paired t-tests, with significant differences assumed at \( P < 0.05 \).

RESULTS

Food intake. The 3V rats in the preplug condition reduced their 30-min intakes of Ensure by 76.5 ± 6.9% after CART, compared with saline control (\( P < 0.01 \); Fig. 1). The CART-induced reduction in intake was significant by 3 min (\( P < 0.05 \)). After the aqueducts were blocked, 3V CART still inhibited intake, but the
magnitude of inhibition was significantly attenuated (26.1 ± 10.3% feeding suppression, P ≤ 0.05; CART preplug vs. CART postplug, P = 0.01), and the suppression of food intake did not become significant until the 12-min time point (P ≤ 0.05) [3V 30-min intake: drug effect, F(1,9) = 40.993, P = 0.0001; plug effect, F(1,9) = 55.408, P = 0.0001; and drug + plug interaction, F(1,9) = 5.765, P = 0.0398].

The 4V rats reduced their 30-min intakes of Ensure in response to 1 µg of CART, both before (52.8 ± 12.8% feeding suppression, P ≤ 0.01) and after their cerebral aqueducts were obstructed [72.9 ± 7.0% suppression, P ≤ 0.01; 30-min intake: drug effect, F(1,7) = 45.674, P = 0.0003; and drug + plug interaction, F(1,7) = 6.497, P = 0.0382; Fig. 2]. Intakes of Ensure with saline injections were higher after than before blocking the aqueduct, but the suppression of intake was not statistically greater after the plug than before, and intakes with CART treatment were comparable before and after aqueduct occlusion. The anorectic response to CART in the preplug condition was significant at 6 min [P ≤ 0.05; drug, F(1,7) = 22.784, P = 0.002] and by 3 min in the postplug condition [P ≤ 0.01; drug, F(1,7) = 6.415, P = 0.0391].

Other behaviors. The effects of CART on feeding and typical postprandial behaviors, before and after aqueduct blockade, in 3V and 4V rats are shown in Table 1. In the preplug condition, 3V CART reduced counts of feeding (P ≤ 0.05) and grooming (P ≤ 0.05). After the aqueduct was blocked, CART no longer decreased observations of feeding or grooming [feeding: plug, F(1,9) = 8.617, P = 0.0166; drug + plug, F(1,9) = 7.5, P = 0.0229; and grooming: drug, F(1,9) = 14.188, P = 0.0044]. Before the plug, a trend for CART to increase counts of resting did not reach significance nor did a trend for CART to reduce exploratory behavior. An increase in exploratory behavior with CART treatment after aqueduct blockade in 3V rats failed to reach significance [exploring: drug + plug, F(1,9) = 6.348, P = 0.0328; resting: no significant differences].

In 4V rats, both before and after the aqueduct plug, CART decreased the numbers of time points at which we observed feeding behavior [feeding: drug, F(1,7) = 14.538, P = 0.0066; plug, F(1,7) = 27.323, P = 0.0012; Table 1]. Although CART did not significantly reduce grooming in either plug condition, CART tended to reduce grooming overall [drug, F(1,7) = 5.444, P = 0.0524]. There was a tendency for the plug to increase counts of resting, particularly when CART was given.

Table 1. Alterations in rat prandial and periprandial behaviors after 4V or 3V CART before and after Plug

<table>
<thead>
<tr>
<th>No Plug</th>
<th>Plug</th>
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<tr>
<td></td>
<td>3V</td>
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<td>Saline</td>
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<tr>
<td>Feed</td>
<td>2.2 ± 0.2</td>
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<tr>
<td>Groom</td>
<td>1.9 ± 0.4</td>
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<tr>
<td>Rest</td>
<td>3.1 ± 0.9</td>
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<tr>
<td>Explore</td>
<td>3.6 ± 0.7</td>
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<tr>
<td>Feed</td>
<td>1.9 ± 0.3</td>
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<tr>
<td>Groom</td>
<td>1.9 ± 0.5</td>
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<tr>
<td>Rest</td>
<td>4.3 ± 0.7</td>
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<tr>
<td>Explore</td>
<td>1.9 ± 0.4</td>
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Data are expressed in numbers of observations during the 30-min test; means ± SE. *Significant decreases (P ≤ 0.5) by cocaine- and amphetamine-regulated transcript (CART), compared with the accompanying blockade of the cerebral aqueduct (Plug) or no Plug saline control. 4V, fourth ventricular; 3V, third ventricular.
There were no changes in exploring by 4V rats with CART before or after the plug.

Before their aqueducts were obstructed, 3V rats responded to CART with flat-backed and arched-backed postures and tremors that were associated with movement (Fig. 3). These signs were rapid in onset and started with the flat-backed posture within 5 min. This posture was soon accompanied by the tremors, which became more severe as the test progressed. Finally, the flat-backed posture was gradually replaced with an arched-backed posture in some rats by the end of the 30-min observation period. When continuity between the 3V and 4V was disrupted in these 3V rats, there was a significant reduction in the number of time points during the 30-min test at which flat-backed postures and movement-associated tremors were observed after CART. A reduced incidence of CART-induced arched-backed postures in 3V animals with aqueduct blockade did not reach significance (Fig. 3).

In 4V rats, both before and after aqueduct plugs were introduced, CART treatment produced the tremors and altered body postures during the 30-min observation period (Fig. 4). We noticed that the tremors were more intense in 4V rats than in preplug 3V rats. The tremors also lasted up to 6 h after CART treatment in 4V rats with plugs, whereas they were no longer noticeable 1 h after CART injection in 3V rats with plugs. The postural changes and tremors were never seen in rats after saline injections.

**DISCUSSION**

These data indicate that tissues caudal to the cerebral aqueduct mediate the major anorectic effect of intracerebroventricular CART. When CART had access to the 4V, either by direct injection (Fig. 2) or by secondary flow from the 3V (“3V CART” in Fig. 1), robust and rapid reductions in food intake were observed. When an aqueduct plug prevented 3V CART from reaching the 4V, CART’s hypophagic effect was postponed and significantly attenuated (Fig. 1). Although CART in the 3V reduced food intake somewhat when aqueduct plugs were in place, this may not have been due to a site of action near the 3V, but rather could be explained if CART leaked past the plugs in a few 3V animals. This was indicated by data from three rats whose food intakes were significantly lower than those of the remainder of the group. Without these three rats, the group would have reduced its intake by only 9.8 ± 8.6% (n = 7) instead of 26.1 ± 10.3% (n = 10). These rats also exhibited motor signs that were otherwise seen only when CART was intended to reach the 4V. We evaluated the effectiveness of aqueduct blockade by examining ink locations immediately after injecting ink intracerebroventricularly. According to this approach, the three rats in question qualified for inclusion in the statistical analysis. Ink might have been apparent beyond the plug in these animals if we had waited 15–30 min to examine the ink placements. Regardless, a difference in hypophagic responses to CART before and after aqueduct blockade in the 3V rats was clear, and we conclude that intracerebroventricular CART exerts its major anorectic effect in the hindbrain. We need to be clear that our interpretation is based on a single dose of CART. However, this dose produces 76.5 and 52.8% reductions in food intake when injected into the 3V or 4V, respectively.

After eating meals, rats typically groom and rest, in what is known as a behavioral satiety sequence (3, 11). In general, the hypophagic effect of intracerebroventricular CART that reached the 4V did not appear to be associated with increased incidences of these behaviors (Table 1), nor with an earlier onset of the sequence. The decrease in postprandial grooming with 4V CART could well result from the diminished food intake. Thus the present behavioral results do not provide evidence for a satiety-enhancing action of CART. This is consistent with our earlier work, in which intracerebroventricular CART neither shortened meal duration nor accelerated the decay of lick rates during liquid diet meals (2) and thus did not appear to accelerate within-meal satiety.

CART’s anorectic action was associated with changes in motor behavior. When CART had access to
the 4V, either directly (Fig. 3) or by secondary flow from the 3V (Fig. 4), we observed tremors and altered postures. When 3V CART was prevented from reaching the 4V, the incidences of these signs were significantly reduced (Fig. 4). Therefore, both the anorexigenic and gross motor effects of CART appear to be mediated by sites in the caudal hindbrain. The fact that 4V CART produces both anorexia and tremors raises the important question of whether the anorexia and the motoric effects are somehow linked. Several alternative hypotheses arise from our data: 1) 4V CART produces anorexia by acting at a hindbrain site different from that which is involved in the motoric effects; 2) 4V CART produces tremors and other motor alterations, which then interfere with normal ingestion; or 3) 4V CART acts at a site in the hindbrain to produce a phenomenon that leads to both the anorexia and the motor signs separately. Further work is needed to distinguish among these possibilities. CART injected directly into the ventral tegmental area increases locomotor activity and produces a conditioned place preference (16), lending support to the notion that CART can have specific behavioral effects at particular brain sites.

The rapidity with which intracerebroventricular CART produced its hypophagic and motoric effects suggests periventricular sites of action. CART fibers have been demonstrated in hindbrain sites that surround the 4V and that play important roles in the control of feeding, including the nucleus of the solitary tract, parabrachial nucleus, locus coeruleus, and raphe nuclei (19). The flat-backed and arched-backed postures and movement-associated tremors we observed seem similar to some of those described for serotonin syndrome in rats. Serotonin syndrome can be produced experimentally under conditions of enhanced serotonergic neurotransmission and serotonin-receptor supersensitivity. In addition to the motor disturbances we report here, the syndrome has also been commonly reported to include forepaw treading, lateral head weaving, hindlimb abduction, Straub tail, muscle rigidity, and autonomic signs such as salivation, piloerection, ejaculation, and hyperpyrexia (4, 12, 13, 15). We did not specifically look for all of these signs. However, the timing and order of appearance of the altered postures and motor disturbances that we do report are consistent with those seen previously in rats with serotonin syndrome (4) and may be consistent with the reductions in normal exploratory and grooming behaviors seen in rats in this condition (24). These similarities suggest that CART in the 4V may activate serotonergic raphe neurons to enhance the release of serotonin and produce changes in motoric function, perhaps at spinal motoneurons (9).

Perspectives

The rapid acceptance of CART as a neuropeptide with relevance for feeding and energy balance was fueled by early demonstrations that CART is localized in brain regions known to play roles in these functions and by anatomic relationships of CART to leptin receptors and to other neuropeptides in the hypothalamus. Subsequent studies showed that lateral ventricular CART increases c-fos expression in brain structures involved in feeding and reduces food intake. These results justified further investigation into potential roles for CART in the control of food intake, but it would have been premature to draw conclusions about specific roles for CART based on the early work. The emerging evidence from this laboratory questions the specificity of CART and lays important groundwork for a focus on potential hindbrain mechanisms involved in the effects of CART. Attention to the behavioral status of the animal is critical to these analyses. For example, the tremors produced by CART, mentioned in passing in previous reports, may provide clues about brain sites and neurochemicals that are responsive to CART. Thorough behavioral analysis will continue to be an important adjunct to molecular biological data in the study of CART, and of other neuropeptides yet to be discovered, in the control of energy balance and food intake.

National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-19302 supported this research.

REFERENCES


