Intracerebroventricular CART peptide reduces rat inge- 

tive behavior and alters licking microstructure

SUSAN AJA,1 GARY J. SCHWARTZ,1 MICHAEL J. KUHAR,2 AND TIMOTHY H. MORAN1
1Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205; and 2Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia 30329

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Aja, Susan, Gary J. Schwartz, Michael J. Kuhar, and Timothy H. Moran. Intracerebroventricular CART peptide reduces rat ingestive behavior and alters licking microstructure. Am J Physiol Regulatory Integrative Comp Physiol 280: R1613–R1619, 2001.—Intracerebroventricular administration of cocaine- and amphetamine-regulated transcript (CART) peptides reduces food intake and increases c-Fos in brain areas involved in the control of feeding. To discern behavioral mechanisms through which CART alters the microstructure of feeding, we injected CART-(55–102) (0.1, 0.5, 1, 2 μg, and saline controls) into the lateral ventricle of male Sprague-Dawley rats 5 min before dark onset and, using lickometers, monitored the ingestion of an Ensure liquid diet for the first 6 h of dark. At a threshold dose of 1 μg, CART dose dependently 1) decreased intake of Ensure in licks; 2) decreased meal size, but did not alter meal duration or number; 3) reduced initial lick rate of meals; and 4) significantly reduced burst number, licks/burst, and licks/cluster. CART dose dependently increased inter lick interval (0.5 μg threshold, 192 ± 4 vs. 183 ± 3 ms, control; 1 μg: 201 ± 1 ms; 2 μg: 214 ± 6 ms). These data suggest that altered oral motor function, and possibly palatability perception, may be fundamental to the anorexigenic action of CART.

hypophagia; peptides; hypothalamus; hindbrain

PEPTIDES ARISING FROM A NEWLY recognized cocaine- and amphetamine-regulated transcript (CART) have received recent attention as putative modulators of food intake. CART mRNA was originally isolated from rat striatum by PCR differential display and found to be regulated transcriptionally by acute exposure to cocaine or amphetamine (10). Two forms of CART mRNA have been identified in the rat brain. The short mRNA encodes a peptide 116 amino acids in length with a hydrophobic leader sequence of 27 amino acids, resulting in a mature peptide 89 amino acids in length. The longer mRNA gives rise to a peptide with an additional 13 amino acid insert in the NH2 terminal region, resulting in a mature form with 102 amino acids (10). Based on its hydrophobic leader sequence and the presence of several basic amino acid pairs commonly found in propeptides, some have proposed that CART is a neuropeptide that is secreted and processed (22, 31). Tissue-specific processing of CART has been demonstrated, with different peptide segments naturally occurring in the pituitary, nucleus accumbens, hypothalamus, and adrenal glands of the rat (18, 32).

CART mRNA (6) and immunohistochemically detected peptide fragments (17, 18) are abundantly expressed in areas of the rat brain involved in sensory processing, stress, reward, and the control of feeding behavior (20). Notably, CART is expressed in hypothalamic regions thought to be important in the control of food intake, including the dorsal, ventromedial, lateral, paraventricular, and arcuate nuclei (6, 18, 19).

Recombinant CART peptide from the short rat mRNA [rsCART-(42–89)] (19, 36) and other CART fragments (21) that contain the carboxy terminal potentially inhibit intake of solid food when administered into the lateral ventricle. rsCART-(42–89) given intracerebroventricularly (icv) also induces c-Fos expression in areas of the rat brain that are involved in feeding behavior, including the hypothalamus, amygdala, parabrachial nucleus, and the nucleus of the solitary tract (36). Evidence for a specific role for endogenous CART in the control of food intake comes from the observation that icv antibodies to CART stimulate feeding (19, 21).

In the present study we tested the effects of a synthesized CART peptide on intake, meal patterns, and licking microstructure during ingestion by rats of a nutritionally complete liquid diet. Meal pattern analysis can reveal behavioral correlates of the initial acceptability of the diet and of the strength and time course of potential negative feedback signaling on continued ingestion, specifically postingestive consequences that may help determine intermeal interval and subsequent meal size and number. Testing with liquid diets also permits microstructural analysis of the ingestive act itself and comparisons with other well-defined manipulations that affect food intake.

METHODS

Male Sprague-Dawley rats (225–250 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. After the animals had adapted to 1 wk of daily,

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Address for reprint requests and other correspondence: S. Aja, Johns Hopkins School of Medicine, Dept. of Psychiatry, 720 Rutland Ave., Ross 618, Baltimore, MD 21205 (E-mail: susanaja@hotmail.com).

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scheduled 30-min access to vanilla-flavored Ensure (1.05 kcal/ml; Ross Laboratories, Columbus, OH) during the light phase, we identified animals that ingested at least 8 ml of Ensure during the test.

The rats (n = 13, 315.1 ± 3.8 g at surgery) were then stereotaxically implanted with unilateral stainless steel cannulas aimed at the lateral ventricle. For this 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 level skull position. A hole was then drilled in the skull 1.0 mm caudal to bregma and 1.3 mm lateral to midline, and a 23-gauge stainless steel guide cannula was lowered to 5.0 mm below dura. The cannula was secured in place with dental cement and stainless steel screws implanted in the skull. A 30-gauge stainless steel obturator was inserted into the cannula to maintain patency. Rats were given penicillin (300,000 units/ml, 0.2 ml im) to prevent postoperative infection.

After 1 wk of postoperative recovery, cannula placements were assessed by examining water intake in responses to icv angiotensin II. For this test, rats were deprived of water for 1 h, injected with angiotensin II (50 ng/5 µl) or 0.9% sterile saline vehicle icv, and allowed 30 min access to water in graduated drinking tubes. Eight rats whose water intake after angiotensin II was at least 5 ml greater than their intake after saline injection were selected for housing in the lickometer cages. Rats were adapted to food deprivation during the final 3 h of light, followed by 6-h lickometer-monitored Ensure access beginning at lights out. The subsequent 6–21 h volumetric measurement of Ensure intake was not monitored by the lickometer.

We used a CART peptide synthesized from the long form of rat CART mRNA [rCART-(55–102), American Peptide, Sunnyvale, CA]. Rats were injected with CART (0.1, 0.5, 1, or 2 µg/5 µl) or sterile 0.9% saline vehicle icv in the lateral ventricle just before lights out and Ensure access. The effects of CART on Ensure intake, meal patterns, and licking microstructure were assessed on test days separated by at least 1 day with no injection. One dose of CART and an accompanying saline control were tested each week in descending dose order. Injections were made with a Gilmont microliter syringe attached to polyethylene tubing and a 30-gauge stainless-steel injector that extended 1.5 mm past the tip of the guide cannula. At the end of the experiment we verified cannula patency by again examining water intake in response to angiotensin II.

Meal patterns and microstructural analysis of Ensure intake. Lickometer cages were equipped with graduated drinking bottles with stainless steel drinking tubes attached to an interface (Di LOG Instruments and Systems, Tallahassee, FL) that passed <60 nA of current through the rat each time its tongue made contact with the drinking tube. The current was amplified and sent to an IBM AT computer that recorded the time of each tongue contact to the nearest millisecond. Data files were transferred to diskettes for analyses with the Tongue Twister program (16).

We analyzed the data according to an approach previously described in detail (9). In brief, lickometric data from the first meal and from the entire 6-h test were analyzed to quantify numbers of licks, bursts and clusters, licks/burst, licks/cluster, the average length of the interlick interval, and latency to the first meal. For our analysis, all nonburst licks were filtered. The criterion for the end of a burst of licking was an interlick interval between 230 and 500 ms. The criterion for the end of a cluster was an interlick interval of 500 ms or longer. In addition, the number of licks was calculated for each animal in 1-min intervals. To quantify changes in rate of licking during meals, we fit these data to a Weibull function, \( y = A \exp\left[-(Bt)^C\right] \). This function has been used by Davis and colleagues (7) because it has the theoretical significance of an exponential function (8) and has been shown to fit satiation curves well (23). The A parameter is the initial lick rate in licks/min, B is the rate of decay of licking in licks/s, and C is a dimensionless shape parameter, a measure of how closely the function resembles an exponential. If C = 1, the Weibull function is a simple exponential curve. When C > 1, the initial rate of decay is less rapid than it would be for an exponential.

Meals were defined as beginning with three licks separated by interlick intervals less than 250 ms and followed by 5 min or longer without licking. Meal data were analyzed for meal size (number of licks), meal number, intermeal interval (min), and satietiy ratio (intermeal interval divided by meal size, multiplied by 100). Data for all variables were analyzed by repeated-measures ANOVA with planned paired t-tests. Significant ANOVA and group differences were assumed at \( P \leq 0.05 \).

### RESULTS

ANOVA showed that icv CART significantly reduced intake of Ensure, in licks, during the 6-h test (Fig. 1, Table 1; drug: \( F_{1,7} = 47.171, P = 0.0002 \); drug × dose interaction: \( F_{3,21} = 3.102, P = 0.0486 \)). The 1-µg dose was threshold for this anorectic effect, reducing the number of licks by 28.7% (\( P = 0.05 \)), whereas 2 µg CART reduced licks by 55.9% (\( P = 0.01 \)). Drug-related attenuations of burst number (\( F_{1,7} = 7.032, P = 0.0386 \)), licks/burst (\( F_{1,7} = 7.513, P = 0.0289 \)), and licks/cluster (\( F_{1,7} = 6.459, P = 0.0329 \)) were measured during the 6-h test (Table 1). CART's reduction in numbers of licks was due to drug-related reductions in meal size (Fig. 2, Table 2; drug: \( F_{1,6} = 50.307, P = 0.0004 \); drug × dose: \( F_{3,18} = 4.388, P = 0.0174 \)). The threshold dose of 1 µg of CART reduced licks per meal by 31.3%, and 2 µg of CART reduced meal size by 65%. CART did not alter the number of meals taken during the test or the mean meal duration (Table 2). Although the intermeal interval with 2 µg of CART was signifi-
different from its saline control, it did not differ from responses to lower doses of CART. The decreased average meal size with 2 \( \mu g \) CART resulted in a high satiety ratio (Table 2).

Weibull analysis of lick rates during meals over the entire 6-h test revealed that CART reduced the initial lick rate (Fig. 3, Table 2; drug: \( F_{1,6} = 42.996, P = 0.0006; \) dose: \( F_{3,18} = 9.131, P = 0.0007; \) dose \& drug: \( F_{3,18} = 24.122, P < 0.0001 \)) by 34\% with 1 \( \mu g \) and by 64\% with 2 \( \mu g \) of CART. CART did not affect the decay of lick rate or significantly alter the shape parameter (Table 2). Weibull functions, constructed with averages of initial lick rates, licking decays, and shape parameters for meals during the 6-h test (Fig. 4, 2 \( \mu g \) CART and saline control shown), demonstrate that CART reduced initial lick rate but not the rate of decline of licking during meals.

CART increased the average length of the interlick interval dose dependently (Fig. 5; drug: \( F_{1,7} = 35.847, P = 0.0005; \) dose: \( F_{3,21} = 9.334, P = 0.0004; \) drug \& dose: \( F_{3,21} = 6.942, P = 0.002 \)). The threshold dose of CART for prolonging the interlick interval was 0.5 \( \mu g \) (192 ± 4 vs. 183 ± 3 ms control, \( P = 0.05 \)). CART produced a dose-dependent rightward shift in the distribution of the within-burst interlick intervals illustrated in Fig. 6 for 2 \( \mu g \) of CART, compared with the normal distribution of its saline control.

CART's overall effects were also evident in the first meal. CART significantly decreased the initial lick rate of the first meal in a dose-dependent manner (drug: \( F_{1,4} = 21.309, P = 0.0099; \) drug \& dose: \( F_{3,12} = 10.715, P = 0.001 \)). The threshold dose of 1 \( \mu g \) reduced initial lick rate by 35.2\% (130.8 ± 27.0 vs. 201.2 ± 19.8 licks/min, saline control, \( P = 0.01 \)), and 2 \( \mu g \) of CART caused a 65\% reduction (166.1 ± 26.2 vs. 188.9 ± 12.5 licks/min, saline control, \( P = 0.01 \)). CART did not significantly alter the decay rate or the shape parameter for the first meal.

Other lickometric parameters that were altered by CART during the 6-h test were altered during the first meal at the 2-\( \mu g \) dose only. Latency to the first lick was not significantly affected overall (drug \& dose: \( P = 0.1187 \)), but when 2 \( \mu g \) CART was tested, three rats did not initiate licks until 30–60 min into the test, and one rat did not lick until the 6-h lickometer test was almost over (3712.6 ± 2467.3 vs. 2.7 ± 1.7 s, saline; \( P < 0.05 \)). The 2-\( \mu g \) dose of CART decreased the size of the first meal (665.4 ± 230.3 vs. 2114.0 ± 308.7 licks, saline; drug: \( F_{1,6} = 23.77, P = 0.0028; \) drug \& dose: \( F_{3,18} = 3.192, P = 0.0486 \)) by reducing licks/cluster (14.3 ± 3.6 vs. 32.6 ± 3.4 licks/cluster, saline; drug: \( F_{1,7} = 14.957, P = 0.0062 \)), and number of bursts (61.9 ± 17.2 vs. 139.9 ± 12.6 bursts, saline; dose: \( F_{3,21} = 4.146, P = 0.0181 \)), but not licks/burst. This dose also significantly increased the interlick interval (210 ± 7 vs. 188 ± 4 ms control, \( P = 0.05 \)).

Although motor behaviors other than licking were not formally measured in this study, we noticed a variety of behavioral alterations in response to the 1- and 2-\( \mu g \) doses of CART. CART produced a trance-like state, flat-backed and arched-backed postures, cage licking, and movement-associated tremors of the head and, in more severe cases, the entire body.

**DISCUSSION**

Our data show that synthesized rlCART-(55–102) reduces licking behavior and alters liquid diet meal patterns and lick microstructure. The results are con-
cistent with actions that may be secondary to reduced perception of palatability and/or alterations in motoric function.

CART significantly reduced 6-h licking behavior at doses of 1 and 2 μg (Fig. 1) due to a specific reduction in meal size rather than in any change in meal number (Fig. 2, Table 2). A number of peptides with proposed or demonstrated roles in satiety reduce food intake through relatively specific effects on meal size. For example, cholecystokinin (CCK) administered at meal onset results in consistent decreases in meal size (38), whereas blockade of CCK receptors results in increases in meal size (24, 30). Similarly, leptin-induced anorexia is characterized by specific reductions in meal size, whereas the hyperphagia with leptin signaling deficits is the result of increases in meal size. Interpretations arising from CART’s ability to inhibit food intake have focused on hypothalamic CART (18, 19, 21, 35, 36), and CART produced in the arcuate nucleus has been postulated to mediate some of the anorexigenic effects of leptin. Leptin may decrease food intake, in part, by downregulating the production of mRNAs for neuropeptide Y and agouti-related peptide (11, 26, 27) in one population of neurons in the arcuate nucleus (5) and upregulating the expression of mRNAs for CART (19) and proopiomelanocortin (28, 33) in a separate set of arcuate neurons (35). CART production is decreased in leptin-deficient ob/ob mice after starvation and increased after leptin treatment (13, 19, 25, 33), and the fall in leptin with fasting results in a decrease in CART mRNA (1). The present data demonstrating that CART, like leptin, reduces food intake by altering meal size appear consistent with this overall view of a role for CART in the mediation of anorexic actions of leptin.

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Data are means (SE). *Significant drug effect, †significant dose effect, †significant drug × dose interaction; CART significantly different from saline control, †P ≤ 0.05, †P ≤ 0.01. Satiety ratio, (IMI/meal size × 100). Weibull parameters: initial licks, licks/min; decay, s⁻¹; shape, dimensionless.

Fig. 3. icv CART-induced reductions in initial lick rates for meals during 6-h access to Ensure. Data are expressed in licks/min, means ± SE. Significant differences between CART treatment and corresponding saline control: †P ≤ 0.01.

Fig. 4. Weibull functions of within-meal licking rates, in licks/min, after 2 μg of icv CART and corresponding saline control.
Unlike other peptides that reduce meal size, CART did not significantly alter meal duration. The microstructural data indicated that CART’s action on reducing meal size was through a reduction in the initial rate of licking rather than through alterations in the onset or satiety. CART affected neither the rate of licking decay during meals nor the shape parameter. Thus the Weibull functions for doses of CART that altered meal size differ from functions for saline controls in initial lick rate only and are otherwise almost identical in shape (Fig. 4). Despite these results, CART is appropriately positioned to modify sensory input to the brain from meal-related stimuli on the control of ingestion patterns (4, 17).

CART decreased the average initial lick rate of meals dose-dependent (Fig. 3, Table 2), and this effect was seen as early as the first meal. Traditionally, reductions in initial lick rate have been interpreted to reflect diminished initial acceptability of the ingestate (3, 8). The presence of CART in fibers localized in areas that receive, process, and integrate taste (nucleus of the solitary tract, parabrachial nucleus, and amygdala) and olfactory information (olfactory bulb and cortex, amygdala, and thalamus) (17) are consistent with a potential action for CART in altering the perceived palatability of food.

However, the data on average interlick interval suggest that the decreased initial lick rate may not simply reflect CART-induced changes in perceived palatability. CART increased the average interlick interval dose-dependently and substantially during the 6-h test (Fig. 5) and had early effects at the highest dose we tested. In the rat, each burst is a series of licks at a high constant rate of 6 to 8 licks/s. Because the variability in distribution of interlick intervals during a burst is very small, it has been proposed that licking is controlled by a motor pattern generator in the hindbrain (37). Our data indicate a robust rightward skew in the peak of within-burst interlick intervals between 100 and 250 ms (Fig. 6) that accounts for the substantial lengthening of interlick interval with CART (Fig. 5). Thus icv CART appeared to slow the licking act, rather than cause missed licks. The interlick interval even increased with 0.5 μg of CART during the 6-h lickometer test (Fig. 5), a dose that did not significantly reduce the overall number of licks (Fig. 1) or meal size (Fig. 2). We suggest that modulation of oral motor function contributes to the anorexia and that licking must slow significantly before overall food intake is affected. Thus the increase in interlick interval may be a more sensitive measure of CART effects on ingestion than its overall reduction of number of licks.

Central pattern generators in the brain stem (14) organize licking and mastication. These systems are composed of rhythm-generating neurons that project to oromotor nuclei and neurons that drive the firing pattern, both in the medullary reticular formation (reviewed in Ref. 34). Within the brain stem, nuclei that receive sensory signaling from the oral cavity and gastrointestinal tract, including the nucleus of the solitary tract (29) and the parabrachial nucleus (15), project to the parvocellular reticular formation in the rat. The nucleus of the solitary tract and parabrachial nucleus also have dense representations of CART-immunoreactive fibers (17) and show elevated c-Fos after icv CART (36). Because CART peptides are present in and may be active at brain regions that receive and integrate meal generated signals and that also project to oral motor generator systems, CART has the potential to modify the influences of meal-generated signals on oromotor ingestive patterns.

We noticed a variety of behavioral alterations after the 1- and 2-μg doses of CART, including altered postures and movement-associated tremors. Some of the anorectic effects of icv CART could be secondary to these abnormal behaviors. The ability to initiate meals or bursts appeared prevented by the highest dose of CART, as indicated by a reduction in the number of bursts during the test and by the dramatic increase in latency to the first lick seen in some animals. We do not think that these changes reflect a reduced drive to eat, because even animals with obvious movement-associated tremors approached the drinking spout and attempted to ingest the liquid diet. However, the animals

Fig. 5. icv CART-induced increases in average interlick interval during 6-h access to Ensure. Data are expressed in milliseconds, means ± SE. Significant differences between CART treatment and corresponding saline control: *P ≤ 0.05, †P ≤ 0.01.

Fig. 6. Shapes of histograms that describe the distributions of interlick intervals during 6-h access to Ensure. Responses to 2 μg CART and its saline control are shown for illustrative purposes. Data are expressed as numbers of interlick intervals of different durations (ms).
appeared to have physical difficulties performing the task. Because the highest dose of CART reduced burst size as well as burst number, CART may affect motor capabilities underlying both the beginning of a burst and the ability to continue licking once a burst has begun. Finally, the increases in interlick interval with CART were far greater than those seen with haloperidol, a dopamine receptor antagonist known to cause motor deficit (12). Together, these data suggest that CART’s effects on food intake may be secondary to its effects on overall motoric competence. It remains to be seen if CART’s hypophagic action function can be separated from its effects on oral and whole-body motor function. Selective injections of CART into different ventricles and parenchymal injections of CART into periventricular hypothalamic and hindbrain areas may reveal feeding-specific effects of CART that are obscured by the motoric behaviors that can occur after lateral ventricular injection of this peptide.

**Perspectives**

CART’s localization to hypothalamic nuclei with documented roles in energy balance and its modulation by leptin have both contributed to its wide acceptance as an anorexigenic neuropeptide. However, thorough evaluation of CART’s effects on the microstructure of the animal during feeding informs the data, and behavioral status of mechanisms through which CART may produce its hypophagic effect. Attention to the behavioral status of the animal during feeding informs the data, and behavioral approaches will continue to be essential for the critical evaluation of roles for CART, and anorexigenic agents yet to be discovered, in the modulation of food intake.

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