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Cocaine- and amphetamine-regulated transcript peptide produces anxiety-like behavior in rodents

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Abstract

Cocaine- and amphetamine-regulated transcript (CART) peptide (CART-(55–102)) is involved in the suppression of food intake. We now report that CART-(55–102) is involved in anxiety in rodents. Intracerebroventricularly administered CART-(55–102) as well as intraperitoneal administration of *N*-methyl-β-carboline-3-carboxamide (FG-7142), a selective $GABA_A$/benzodiazepine receptor inverse agonist, reduced time spent in the open arms in the elevated plus-maze task in mice. CART-(55–102)-induced anxiogenic-like behavior in this task was attenuated by widely prescribed anxiolytics such as diazepam and buspirone. Likewise, CART-(55–102) and FG-7142 significantly reduced social interaction in mice. Both diazepam and buspirone significantly reversed CART-(55–102)-induced anxiogenic-like behavior in social interaction tests. By contrast, another biologically active CART peptide, CART-(62–102), was without effect in the elevated plus-maze task in mice. Moreover, intracerebroventricular administration of CART-(55–102) markedly increased the firing rate of locus coeruleus neurons in single unit recording in anesthetized rats. As CART-(55–102) produced anxiety-like effects in rodents, this peptide may possibly be involved in anxiety and stress-related behavior.
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Keywords: CART-(55–102); CART-(62-102); Anxiety; FG-7142

1. Introduction

Cocaine- and amphetamine-regulated transcript (CART) was originally described to be an mRNA acutely upregulated in the nucleus accumbens after administration of cocaine or amphetamine in rats (Douglass et al., 1995). To date, several CART peptide fragments have been identified (Kuhar and Yoho, 1999; Thim et al., 1999). Among them, two CART peptide fragments, CART-(55–102) and CART-(62–102), were isolated and sequenced from the rat hypothalamus and pituitary (Kristensen et al., 1998; Thim et al., 1998, 1999), and found to be biologically active (Kristensen et al., 1998; Bannon et al., 2001). There are several lines of evidence that CART-(55–102) is deeply involved in feeding behavior and energy expenditure (Kristensen et al., 1998; Thim et al., 1998; Wang et al., 2000). Intracerebroventricular administration of CART-(55-102) suppresses food intake in normal and fasted rats (Kristensen et al., 1998; Thim et al., 1998), and induces c-Fos in the brain in the areas related to feeding and energy balance (Vrang et al., 1999, 2000). CART mRNA is also expressed abundantly within hypothalamic structures implicated in the central control of feeding behavior and metabolism, and leptin administration increases CART mRNA in the hypothalamus (Kristensen et al., 1998). In addition to CART-(55–102), CART-(62–105) was also reported to cause a marked reduction in food intake in fasted mice (Bannon et al., 2001).

In addition to abundant expression in the hypothalamus, CART mRNA and CART peptide immunoreactivity are expressed in other brain areas, including the pituitary and limbic systems such as central and basomedial nucleus of amygdala, and septum-hippocampal formation (Couceyro et

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al., 1997; Koylu et al., 1997, 1998). CART-(55–102) administration induces c-Fos expression in the central nucleus of amygdala as well as in the paraventricular nucleus of the hypothalamus (Vrang et al., 1999, 2000). Moreover, in paraventricular nucleus, 89% of cortocotropin-releasing hormone (CRH)-immunoreactive neurons contain c-Fos after i.c.v. injection of CART-(55–102), and CART-(55–102) markedly induces plasma adrenocorticotropic hormone (ACTH) and corticosterone levels (Stanley et al., 2001; Vrang et al., 2000). CART-(55–102) also increases release of CRH from hypothalamic explants (Stanley et al., 2001). These findings indicate that CART-(55–102) may activate the hypothalamus–pituitary–adrenal axis, possibly by interacting with the CRH system, and that CART peptides may be involved in emotional regulation and stress responses. Both CART-(55–102) and CART-(62–102) have been reported to have multiple physiological roles in the central nervous system other than the regulation of food intake (Bannon et al., 2001; Kimmel et al., 2000; Matsumura et al., 2001; Okumura et al., 2000), and another fragment of CART peptides was reported to induce anxiogenic-like activity in rats (Kask et al., 2000). In the present study, we investigated the involvement of CART-(55–102) and CART-(62–102) in anxiety in rodents.

2. Materials and methods

2.1. Materials

Male ICR mice (25–36 g, Charles River, Japan) were housed 10 per cage. Male Wistar rats (300–400 g, Charles River, Japan) were housed three per cage, and used for electrophysiological study. All the animals were maintained under a 12-h light/dark cycle (light on 7:00 a.m.) in a temperature- and humidity-controlled holding room. Food and water were available ad libitum. In experiments for i.c.v. infusion, rats were surgically equipped with a single cannula placed above the lateral ventricle. Animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.), and placed in a stereotaxic apparatus (Narishige, Tokyo, Japan) where a 7-mm-long, 23-gauge stainless steel guide cannula was placed to within 1 mm of the ventricle and anchored to the skull with screws and dental cement. The implantation coordinates were 1.0 mm posterior to the bregma, 1.2 mm lateral to the midline, and 4.5 mm ventral to the cortical surface according to the rat brain atlas of Paxinos and Watson. After a 7-day postsurgical recovery period, cannula patency was confirmed by gravity flow through an 8-mm, 30-gauge injector inserted through the guide to 1 mm beyond its tip. All studies have been reviewed by the Taisho Pharmaceutical Animal Care Committee and have met the Japanese Experimental Animal Research Association standards as defined in the *Guidelines for Animal Experiments* (1987). Rat CART-(55–102) and rat CART-(62-102) were purchased from Peptide Institute (Osaka, Japan), and dissolved in saline containing 0.1% bovine serum albumin and 0.02% KCl. Diazepam and *N*-methyl-β-carboline-3-carboxamide (FG-7142) were dissolved in 0.3% Tween 80/saline, and buspirone was dissolved in saline.

2.2. Elevated plus-maze task in mice

The apparatus consisted of a plus-shaped maze elevated 38.5 cm from the floor and comprising two opposite open arms, 30 × 5 cm, crossed at right angles by two arms of the same dimensions enclosed by 20-cm-high walls with an open roof. In addition, a 0.3-cm-high edge made of Plexiglas surrounded the open arms to avoid falls. Luminosity measured at the center of the maze was 60 lx. During observation, the experimenter always sat in the same place, next to the apparatus. The animals were individually tested in 5-min sessions in the apparatus described above. Each mouse was placed in the center of the plus-maze facing one enclosed arm. The amount of time spent in open arms of the maze was recorded. Mice were naïve to the apparatus. To determine pretreatment time, CART-(55–102) was injected i.c.v. 10, 30 and 60 min prior to the test. Because we observed a significant effect at 30 and 60 min, CART-(55–102) or CART-(62–102) was administered i.c.v. 60 min prior to the test. FG-7142 was administered i.p. 30 min prior to the test. Diazepam and buspirone were administered i.p. 30 min prior to CART-(55–102) administration. The doses and the pretreatment time of these drugs were determined by our previous works and literatures.

2.3. Social interaction test

When two mice from separated cages are placed together in a small chamber in which neither has established territory, they engage in a social interaction which includes a variety of behavioral patterns: sniffing, following, grooming, kicking, crawling under or over the partner, and touching or nearly touching their faces (File and Hyde, 1978). Social interaction test was performed according to the method described by Beneytez et al. (1998) but with slight modification. Mice were housed in their home cage for 5 days before the experiment. On the day of the experiment, CART-(55–102) and FG-7142 were injected i.c.v. and i.p., respectively, into pairs of mice from different home-cages. After 60 min (CART-(55–102)) and 30 min (FG-7142), the pairs of mice from different home cages were placed together in a transparent polycarbonate cage (length:width:height = 24 × 17.6 × 12.0 cm) with fresh wool litter on the floor. Luminosity measured was 135 lx. The time that mice socially interacted (genital investigation, tail licking, neck licking, facing, trunk licking, following, allogrooming, mounting, crawling under or over the partner, wrestling, boxing, kicking, jumping) was visually measured for 5 min by an observer who was not aware of the treatments. The occurrence of at least one of behaviors was recorded.

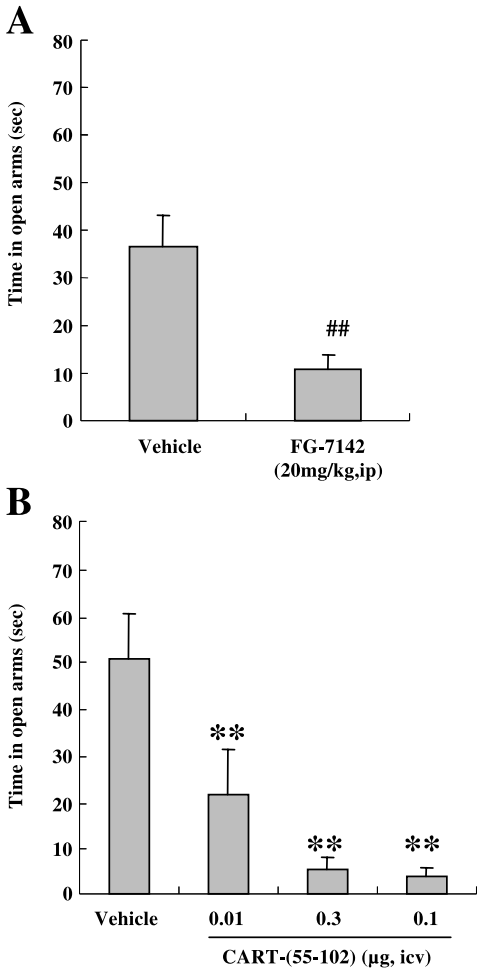

Fig. 1. Effect of FG-7142 (A) and CART-(55–102) (B) on time spent in open arms in elevated plus-maze task in mice. CART (55–102) (i.c.v.) and FG-7142 (i.p.) were administered 60 and 30 min, respectively. Data represent mean ± S.E. $^{\#\#}P<0.01$ versus vehicle (Student's t test). $^{**}P<0.01$ versus vehicle (Dunnett's test).

Diazepam and buspirone were administered i.p. 30 min prior to CART-(55–102) administration.

2.4. Electrophysiological experiments

Animals were anesthetized with urethane (1.5 g/kg, i.p.) and fixed in a stereotaxic apparatus. Body temperature was maintained at 37 ± 1 °C with a heating pad (KN-474, Natsume, Tokyo, Japan). Extracellular single unit recordings of the locus coeruleus neurons were made as previously described (Nakamura, 1977; Okuyama et al., 1999). Stimulating electrodes consisting of two insulated stainless wires with an exposed tip of 0.5 mm were implanted into the dorsal noradrenergic bundle. The same type of electrode was implanted into the frontal cortex to record an electroencephalogram. Coordinates of the dorsal noradrenergic bundle were 1.5 mm anterior to the lambda, 0.8 mm lateral to the midline, and 5.7 to 6.0 mm ventral to the cortical surface, and those for the frontal cortex were 2.0 mm anterior to the bregma, 2.0 mm lateral to the midline, and 1.0 mm ventral to the cortical surface. Stimuli applied to the dorsal noradrenergic bundle were single square pulses of 0.5 to 1 ms with currents ranging from 0.1 to 0.5 mA. The frequency of stimulation was 1 Hz in all experiments. The single-unit activity of locus coeruleus neurons was recorded extracellularly by means of a glass micropipette filled with 2 M NaCl. Location of the locus coeruleus was determined by appearance of field responses evoked by dorsal noradrenergic bundle stimulation. When the tip of a recording electrode was localized correctly in the locus coeruleus, the single-unit activity of locus coeruleus neurons was recorded and was superimposed upon the field response. The i.c.v. cannula for CART-(55–102) infusion was placed, and 1 or 3 μg/5μl of CART-(55-102) was infused over a 3-min period. A CART-(55–102)-induced change in neuronal activity was plotted as a percentage of increase from baseline firing rate. The percentage of increase of each group was calculated.

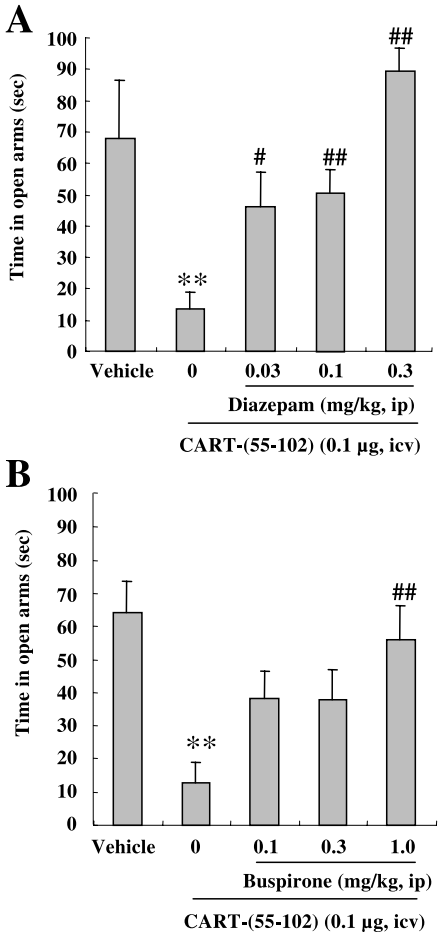

Fig. 2. Effect of diazepam (A) and buspirone (B) on CART-(55–102)-induced reduction of time spent in open arms in elevated plus-maze task in mice. Diazepam and buspirone were administered i.p. 30 min prior to CART-(55–102) injection. Data represent mean ± S.E. $^{**}P<0.01$ versus vehicle (Student's t test). $^{\#}P<0.05$, $^{\#\#}P<0.01$ versus CART-(55–102)-treated group (Dunnett's test).

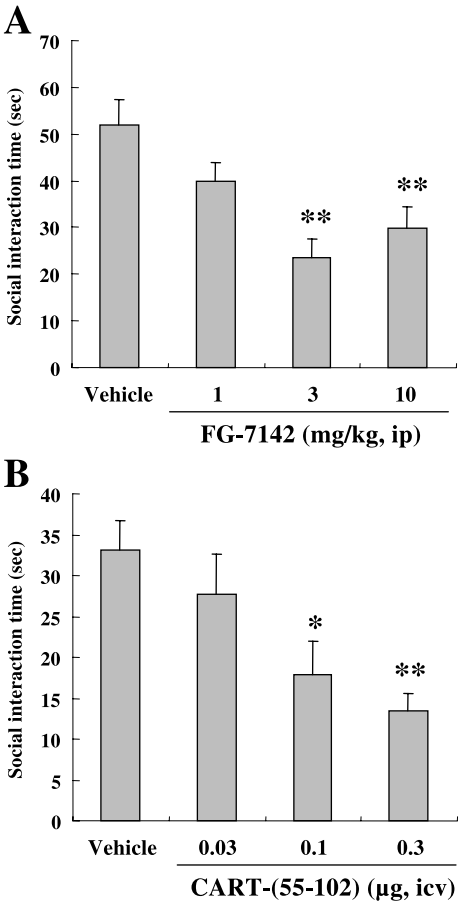

Fig. 3. Effect of FG-7142 (A) and CART-(55–102) (B) on social interaction test in mice. CART-(55–102) (i.c.v.) and FG-7142 (i.p.) were administered 60 and 30 min, respectively, prior to the test. Data represent mean ± S.E. $^{**}P<0.01$ versus vehicle (Dunnett's test).

2.5. Statistical analysis

Data from in vivo experiment were analyzed by one-way analysis of variance (ANOVA) and significant differences between groups were determined using Dunnett's test.

3. Results

3.1. Elevated plus-maze task in mice

The time spent in open arms of the elevated plus-maze was significantly reduced by FG-7142, a selective $GABA_A$/benzodiazepine receptor inverse agonist (Fig. 1(A)). Likewise, intracerebroventricular administration of CART-(55–102) dose-dependently and significantly [$F(3,36)=9.1$, $P<0.01$] reduced the time spent in open arms in the elevated plus-maze task in mice (Fig. 1(B)). The effect of 0.1 μg of CART-(55–102) was reversed by anxiolytics such as diazepam [$F(3,36)=14.6$, $P<0.01$] and buspirone [$F(3,36)=4.4$, $P<0.05$] in a dose-dependent and significant manner (Fig. 2(A) and (B)). Diazepam and buspirone were without significant effects in naïve mice, although diazepam increased the time spent in open arms when higher doses were given (data not shown). In contrast, CART-(62–102) had no effect on time spent in open arms in the elevated plus-maze task (time spent in open arms (s): vehicle, 52.6 ± 11.1; 0.01 μg, 58.5 ± 10.4; 0.1 μg, 52.2 ± 11.0). CART-(55–102) had no effect on spontaneous locomotor activity up to 0.1 μg i.c.v. (counts/120 min: vehicle, 2103 ± 286; CART-(55–102), 2005 ± 436).

3.2. Social interaction test in mice

FG-7142 [$F(3,28)=7.6$, $P<0.01$] as well as CART-(55–102) [$F(3,28)=5.5$, $P<0.01$] caused dose-dependent and significant decreases in the duration of social interaction (Fig. 3(A) and (B)). Reduction in social interaction time was significantly attenuated by diazepam [$F(3,28)=9.4$, $P<0.01$] and by buspirone [$F(3,28)=13.6$, $P<0.01$] (Fig. 4(A) and (B)). Both diazepam and buspirone, at

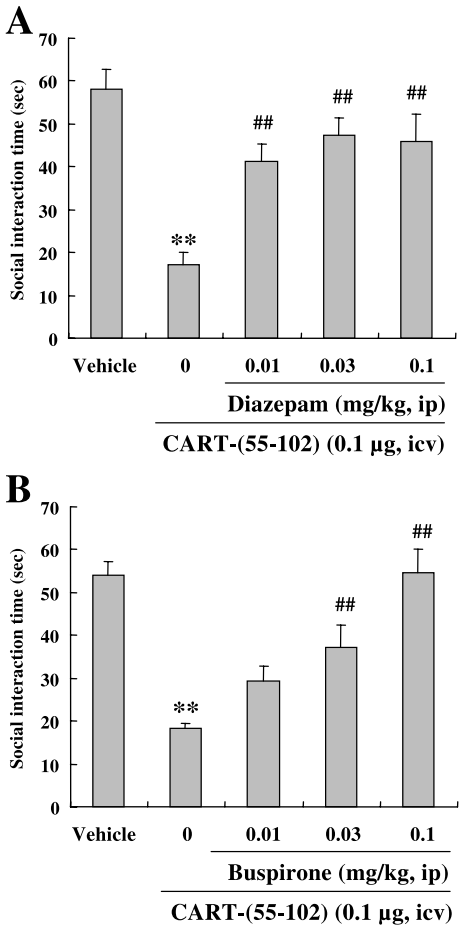

Fig. 4. Effect of diazepam (A) and buspirone (B) on CART-(55–102)-induced reduction in social interaction time in mice. Diazepam and buspirone were administered i.p. 30 min prior to CART-(55–102) injection. Data represent mean ± S.E. $^{**}P<0.01$ versus vehicle (Student's t test). $^{\#\#}P<0.01$ versus CART-(55–102)-treated group (Dunnett's test).

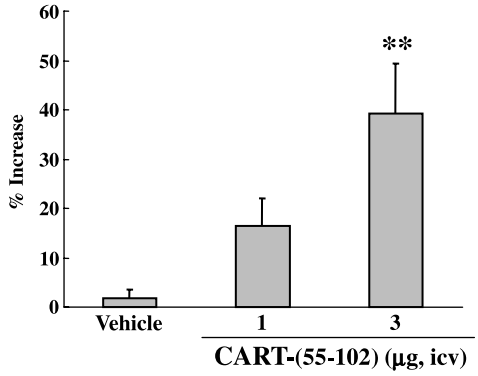

Fig. 5. Effect of CART-(55–102) on firing rate of the locus coeruleus neurons. Data represent mean ± S.E. $^{**}P<0.01$ versus vehicle (Dunnett's test).

doses used in this study, did not affect social interaction in naïve mice.

3.3. Effect of CART-(55–102) on locus coeruleus neuron firing

The firing rate of the locus coeruleus neurons was significantly [$F(2,13)=6.9$, $P<0.01$] increased among the CART-(55–102) (3 μg, i.c.v.)-treated rats compared with findings in the vehicle-treated rats (Fig. 5).

4. Discussion

In the present study, we demonstrated that CART-(55–102) markedly reduced time spent in open arms in the elevated plus-maze test and social interaction time in mice; both are predicative of anxiety-like behavior in rodents. In the present condition, it was confirmed that anxiogenics such as FG-7142 led to anxiety-like behaviors in both elevated plus-maze task and social interactions; hence, both models can serve to measure anxiety-related behavior. At doses of CART-(55–102) that exerted anxiety-like behaviors, CART-(55–102) did not affect spontaneous locomotor activity, showing that the effects of CART-(55–102) occurred independently of changes in locomotor activity. Moreover, anxiogenic-like activity elicited by i.c.v. administration of CART-(55–102) in both elevated plus-maze task and the social interaction test in mice was significantly attenuated by widely prescribed anxiolytics such as diazepam and buspirone. These results strongly support the hypothesis that reduction of time in open arms in the elevated plus-maze task as well as reduction in social interaction time by CART-(55–102) reflect increased anxiety, and that CART-(55–102) may be a potent endogenous anxiogenic peptide.

To date, two CART peptides, CART-(55–102) and CART-(62–102), were isolated from rat hypothalamus and pituitary (Kristensen et al., 1998; Thim et al., 1998, 1999). In the present study, CART-(62–102) had no effect on time spent in open arms in the elevated plus-maze task in mice, even at 0.1 μg. It was reported that CART-(62–102) had physiological effects such as inhibition of food intake, increase in pain threshold and increase in prepulse inhibition at 0.05–0.1 μg i.c.v. (Bannon et al., 2001). We observed the inhibition of food intake after i.c.v. injection of 0.1 μg of CART-(62–102) (data not shown), showing that doses of CART-(62–102) we used in this study are in the biologically active range. Therefore, two biologically relevant forms of CART peptide can be distinguished in vivo based on their potential to cause anxiety in mice. Other than specific effects of CART-(55–102) on the acoustic startle response, this is an interesting example of CART-(55–102) having an effect that was not observed with CART-(62–102), at least at the doses tested. The receptor of CART peptides has yet to be isolated, and it is not known whether receptor subtypes exist. However, it is possible to presume that CART-(55–102) and CART-(62–102) bind to different receptor subtypes, and the receptor involved in anxiety might be different from that involving in feeding. Structural studies on CART peptides showed that N-terminal residues can serve to differentiate the two CART peptides (Ludvigsen et al., 2001). Therefore, the receptors for the two CART peptides probably differ. Another CART peptide fragment, CART-(89–103), was reported to show anxiogenic-like activity in rats (Kask et al., 2000). However, this peptide fragment does not appear to be a naturally occurring CART peptide in rodents.

The mechanism by which CART-(55–102) elicits anxiety-like behaviors in rodents is unknown. It has been reported that CART-(55–102) may interact with CRH neurons, major endogenous mediator to stressful stimuli (Vale et al., 1981). Most CRH-immunoreactive neurons in paraventricular nucleus contain c-Fos after i.c.v. injection of CART-(55–102) (Stanley et al., 2001). Moreover, in a preliminary study, the CART-(55–102)-induced anxiety-like behavior in the elevated plus-maze test was significantly and dose-dependently attenuated by administration of a CRH1 receptor antagonist (unpublished data). These findings suggest that CART-(55–102) may interact with the CRH system, and that this interaction may be involved in the anxiety-like activity elicited by CART-(55–102). This speculation is further supported by the report that the CART-induced inhibition of gastric acid secretion was attenuated by a CRH receptor antagonist through a centrally mediated mechanism (Okumura et al., 2000). It was also reported that CART-(55–102) activates the HPA axis, since CART-(55–102) markedly increases plasma level of ACTH and corticosterone (Vrang et al., 2000). Furthermore, CART mRNA expression is affected by adrenalectomy (Balkan et al., 2001), and, in a preliminary study, we found that CART mRNA in the rat hypothalamus was increased by restraint stress (unpublished data). These findings also suggest that CART peptides increase the activity of the HPA axis, and that CART peptides might be involved in responses to stress.

It was reported that the locus coeruleus, the noradrenergic nucleus, is a key mediator of neurogenic responses to stress (Brady, 1994; Weiss et al., 1994). The locus coeruleus is rich in CRH immunoreactivity (Swanson et al., 1983), and i.c.v. administration of CRH as well as stress increased the locus coeruleus neuronal activity (Okuyama et al., 1999; Valentino et al., 1993). CART is colocalized with tyrosine-hydroxylase in noradrenergic neurons of the locus coeruleus (Koylu et al., 1998). In the present study, i.c.v. injection of CART-(55–102) significantly increased locus coeruleus neuronal activity, as did CRH injection (Okuyama et al., 1999). This finding also indicates that CART-(55–102) might be related to stress responses.

This is the first demonstration that among biologically active CART peptides, CART-(55–102) exerted potent anxiety-like behavior in rodents. Antagonists of CART-(55–102) will be needed to fully address the involvement of CART-(55–102) in emotion-related events and stress-related responses such as anxiety.

References

Balkan, B., Koylu, E.O., Kuhar, M.J., Pogun, S., 2001. The effect of adrenalectomy on cocaine- and amphetamine-regulated transcript (CART) expression in the hypothalamic nuclei of the rat. Brain Res. 917, 15–20.

Bannon, A.W., Seda, J., Carmouche, M., Francis, J.M., Jarosinski, M.A., Douglass, J., 2001. Multiple behavioral effects of cocaine- and amphetamine-regulated transcript (CART) peptides in mice: CART 42–89 and CART 49–89 differ in potency and activity. J. Pharmacol. Exp. Ther. 299, 1021–1026.

Beneytez, M.E., Rodriguez, M.L.L., Rosado, M.L., Morcillo, M.J., Orensanz, L., Fuentes, J.A., Manzanares, J., 1998. Preclinical pharmacology of B-20991, a 5-HT1A receptor agonist with anxiolytic activity. Eur. J. Pharmacol. 344, 127–135.

Brady, L.S., 1994. Stress, antidepressant drugs, and the locus coeruleus. Brain Res. Bull. 35, 545–556.

Couceyro, P.R., Koylu, E.O., Kuhar, M.J., 1997. Further studies on the anatomical distribution of CART by in situ hybridization. J. Chem. Neuroanat. 12, 229–241.

Douglass, J., McKinzie, A., Couceyo, P., 1995. PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. J. Neurosci. 15, 2471–2481.

File, S.E., Hyde, J.R.G., 1978. Can social interaction be used to measure anxiety? Br. J. Pharmacol. 62, 19–24.

Kask, A., Schioth, H.B., Mutulis, F., Wikberg, J.E.S., Rago, L., 2000. Anorexigenic cocaine- and amphetamine-regulated transcript peptide intensifies fear reactions in rats. Brain Res. 857, 283–285.

Kimmel, H.L., Gong, W., Vechia, S.D., Hunter, R.G., Kuhar, M.J., 2000. Intra-ventral tegmental area injection of rat cocaine and amphetamine-regulated transcript peptide 55–102 induces locomotor activity and promotes conditioned place preference. J. Pharmacol. Exp. Ther. 294, 784–792.

Koylu, E.O., Couceyro, P.R., Lambert, P.D., Ling, N.C., DeSouza, E.B., Kuhar, M.J., 1997. Immunohistochemical localization of novel CART peptides in rat hypothalamus, pituitary and adrenal gland. J. Neuroendocrinol. 9, 823–833.

Koylu, E.O., Couceyro, P.R., Lambert, P.D., Kuhar, M.J., 1998. Cocaine- and amphetamine-regulated transcript peptide immunohistochemical localization in the rat brain. J. Comp. Neurol. 391, 115–132.

Kristensen, P., Judge, M.E., Thim, L., Ribel, U., Christjansen, K.N., Wulff, B.S., Clausen, J.T., Jensen, P.B., Madsen, O.D., Vrang, N., Lersen, P.J., Hastrup, S., 1998. Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature 393, 72–76.

Kuhar, M., Yoho, L., 1999. CART peptide analysis by Western blotting. Synapse 33, 163–171.

Ludvigsen, S., Thim, L., Blom, A.M., Wulff, B.S., 2001. Solution structure of the satiety factor, CART, reveals new functionality of a well-known fold. Biochemistry 40, 9082–9088.

Matsumura, K., Tsuchihashi, T., Abe, I., 2001. Central human cocaine- and amphetamine-regulated transcript peptide 55–102 increases arterial pressure in conscious rabbits. Hypertension 38, 1096–1100.

Nakamura, S., 1977. Some electrophysiological properties of neurons of rat locus coeruleus. J. Physiol. (Lond.) 267, 641–658.

Okumura, T., Yamada, H., Motomura, W., Kohgo, Y., 2000. Cocaine-amphetamine-regulated transcript (CART) acts in the central nervous system to inhibit gastric acid secretion via brain corticotropin-releasing factor system. Endocrinology 141, 2854–2860.

Okuyama, S., Chaki, S., Kawashima, N., Suzuki, Y., Ogawa, S., Nakazato, A., Kumagai, T., Okubo, T., Tomisawa, K., 1999. Receptor binding, behavioral, and electrophysiological profiles of nonpeptide corticotropin-releasing factor subtype 1 receptor antagonists CRA1000 and CRA1001. J. Pharmacol. Exp. Ther. 289, 926–935.

Stanley, S.A., Small, C.J., Murphy, K.G., Rayes, E., Abbott, C.R., Seal, L.J., Morgan, D.G., Sunter, D., Dakin, C.L., Kim, M.S., Hunter, R., Kuhar, M., Ghatei, M.A., Bloom, S.R., 2001. Actions of cocaine- and amphetamine-regulated transcript (CART) peptide on regulation of appetite and hypothalamo-pituitary axes in vitro and in vivo in male rats. Brain Res. 893, 186–194.

Swanson, L.W., Sawchenko, P.E., Rivier, J., Vale, W., 1983. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology 36, 165–186.

Thim, L., Nielsen, P.F., Judge, M.E., Andersen, A.S., Diers, I., Egel-Mitani, M., Hastrup, S., 1998. Purification and characterization of a new hypothalamic satiety peptide, cocaine- and amphetamine regulated transcript (CART) produced in yeast. FEBS Lett. 428, 263–268.

Thim, L., Kristensen, P., Nielsen, P.F., Wulff, B.S., Clausen, J.T., 1999. Tissue-specific processing of cocaine- and amphetamine-regulated transcript peptides in the rat. Proc. Natl. Acad. Sci. U. S. A. 96, 2722–2727.

Vale, W., Spiess, J., Rivier, C., Rivier, J., 1981. Characterization of a 41 residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta endorphine. Science 213, 1394–1397.

Valentino, R.J., Foote, S.L., Page, M.E., 1993. The locus coeruleus as a site for integrating corticotropin-releasing factor and noradrenergic mediation of stress responses. Ann. N.Y. Acad. Sci. 697, 173–188.

Vrang, N., Tang-Christensen, M., Larsen, P.J., Kristensen, P., 1999. Recombinant CART peptide induces c-Fos expression in central areas involved in control of feeding behaviour. Brain Res. 818, 499–509.

Vrang, N., Larsen, P.J., Kristensen, P., Tang-Christensen, M., 2000. Central administration of cocaine- and amphetamine-regulated transcript activates hypothalamic neuroendocrine neurons in the rat. Endocrinology 141, 794–801.

Wang, C., Billington, C.J., Levine, A.S., Kotz, C.M., 2000. Effect of CART in the hypothalamic paraventricular nucleus on feeding and uncoupling protein gene expression. NeuroReport 11, 3251–3255.

Weiss, J.M., Stout, J.C., Aron, M.F., Quan, N., Owen, M.J., Butler, P.D., Nemeroff, C.B., 1994. Depression and anxiety: role of the locus coeruleus and corticotropin-releasing factor. Brain Res. Bull. 35, 561–572.