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Central pressor effects of CART peptides in anesthetized rats

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Abstract

Interrelationships between energy homeostasis and regulation of cardiovascular functions have been suggested by previous observations [Am. J. Physiol. 278 (2000) R692; Regul. Pept. 104 (2002) 75; Am. J. Physiol. 277 (1999) R1780]. Cocaine- and amphetamine-regulated transcript (CART) was first discovered in the striatum of rats treated with cocaine or amphetamine. The CART peptides were later found in the hypothalamus and functioned as anorectic peptides. We observed that 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. Intrathecal (levels T2–T3) and intravenous administrations of these peptides, however, showed little or no effects on the heart rate and blood pressure in the rat. Furthermore, an increase of c-Fos-like immunoreactivity in the rat rostral ventrolateral medulla (RVLM) following an I.C. CART 61-102 was observed. The results suggest that central pressor effects of anorectic CART peptides may involve in activation of the medullary sympathetic systems in the rat. Our observations support the hypothesis that energy homeostasis and cardiovascular regulations are closely related and regulated.

Keywords: CART; Blood pressure; Heart rate; Obesity; Intracisternal; Intrathecal

1. Introduction

Cocaine- and amphetamine-regulated transcript (CART) was identified as an mRNA induced in the rat striatum following acute administration of psychomotor stimulant, cocaine or amphetamine (Douglass et al., 1995). The amino acid sequence of CART peptides is highly conserved among species (Douglass et al., 1995; Douglass and Daoud, 1996; Volkoff and Peter, 2001) and a great quantity of CART mRNA was detected in the rat hypothalamus (Gautvik et al., 1996), implying a functional importance of CART. Intracerebroventricular (I.C.V.) injection of a recombinant CART peptide fragment inhibited food intake and completely blocked neuropeptide Y-induced feeding responses while an antiserum against CART peptide enhanced feeding in rats (Kristensen et al., 1998).

The rat CART gene encodes a long and a short form proteins of 129 and 116 amino acids in length, respectively (Douglass et al., 1995). The predicted leader sequence constitutes the first 27 amino acid residues resulting in pro-CART peptides of either 102 (long form) or 89 (short form) amino acids. Several small C-terminal fragments of CART were isolated from rat brain and a tissue-specific proteolytic processing of the proteins was suggested (Thim et al., 1999). CART 42-89 and/or CART 49-89 of the short form (identical to CART 55-102 and CART 62-102 of the long form, respectively) were purified from hypothalamus, nucleus accumbens and pituitary gland by Thim et al. (1999) and both fragments were found to be biologically active in regulating feeding behaviors (Kristensen et al., 1998), several other behavioral responses (Aja et al., 2001;

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Kask et al., 2000; Kimmel et al., 2000; Bannon et al., 2001), gastric functions (Asakawa et al., 2001; Okumura et al., 2000), blood pressure (Matsumura et al., 2001), calcium channel activity (Yermolaieva et al., 2001), and hypothalamo-pituitary axes (Stanley et al., 2001). Other biologically active CART fragments include CART 1-89 and CART 10-89 of the short form isolated from rat adrenal gland (Thim et al., 1999), two dominating fragments CART 54-102 and CART 61-102 produced in yeast (Thim et al., 1998), and CART 55-76 and CART 62-76 of the long form (Lambert et al., 1998; Kask et al., 2000). It is possible that different CART peptide fragments present in various brain nuclei and exert different functions. Most studies have been focused on investigating the physiological functions of the CART peptides using fragment CART 55-102.

A positive correlation between obesity and elevated blood pressure has been confirmed in many epidemiological studies as reviewed by Martínez and Sancho-Rof (1993). While the pathogenesis of obesity-related hypertension and the underlying mechanisms linking these morbid conditions remains obscure. The authors and former colleagues have hypothesized that obesity-related hypertension may be associated with a pathological status of obesity-related signaling molecules (Chen et al., 2000). In addition to inhibiting food intake, I.C.V. CART peptide was also found to increase arterial pressure (AP) and heart rate (HR) in conscious rabbits (Matsumura et al., 2001) indicating the CART peptide might be one of the neuronal signals linking obesity and hypertension. However, the sites of action through which the CART peptide elicits the cardiovascular effects have not been defined. The presence of CART-immunoreactive cells and fibers at a high density in the regions of rostral ventrolateral medulla (RVLM), adrenergic C1 (Koylu et al., 1998), which is believed to be the principal regulators of the arterial blood pressure in the brain by generating tonic sympatho-excitatory activity (Dampney, 1994), provides an anatomical substrate for a role of CART peptides in modulating medullary sympathetic output to the cardiovascular system. In addition, elevated sympathetic activity appears pivotal to obesity-related hypertension (Macdonald, 1995). However, the mechanism underlying sympathetic over-excitation in obese subjects has not been fully discovered. The aim of this study is not only to explore the physiological roles of the CART peptides in the regulation of cardiovascular functions but also to examine a possible involvement of the brainstem in mediating the cardiovascular regulatory effects of CART peptides applied by intracisternal (I.C.) to produce a high concentration locally in the brainstem of rats. Additionally, the expression of the immediate early gene, c-fos, will be detected by immunohistochemical studies to assess the activation and involvement of RVLM neurons following I.C. application of CART peptides and to evaluate the role of CART peptides in regulating brainstem cardiovascular sympathetic outflow.

We here examined the involvement and activity of the naturally occurring hypothalamic CART fragment, CART 55-102 of rat long form pro-CART peptide in the regulation of cardiovascular functions in rats. Since little is known regarding to the cardiovascular activity of other fragments, we also examine the effects of CART 61-102 of rat long form pro-CART peptide. In order to understand the structure–activity relationship of the CART peptide fragments, the effects of CART 61-102 and CART 55-102 in regulating cardiovascular functions will be compared.

2. Materials and methods

2.1. Chemicals and reagents

CART 61-102 and CART 55-102, fragments of rat long form pro-CART peptide, were purchased from Phoenix Pharmaceuticals Inc. (Belmont, CA). Rabbit polyclonal c-Fos antiserum and Fos peptide were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA) and all other reagents for immunohistochemistry were from Vector Laboratories, Inc. (Burlingame, CA). All other chemicals were from Sigma Chemicals Co. (St. Louis, MO).

2.2. Animals

Male Sprague–Dawley rats, weighing 288 ± 47 g (mean $\pm$ SD, $n = 45$), were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Animals were housed in a room with light cycles of 12-h light and 12-h dark, proper temperature and humidity. Animals accessed food and water freely. The use of animals was approved by the Institutional Animal Care and Use Committee of Taipei Medical University.

2.3. Measurements of arterial pressure and heart rate

The rats were anesthetized with urethane of 1.5 g/kg administered intraperitoneally (I.P.). The left femoral artery was cannulated with a polyethylene (PE)-50 tube containing heparinized (10 U/ml) saline. The cannula was connected to a pressure transducer with its output signals to a data acquisition system Biopac MP-30 from Biopac Systems, Inc. (Santa Barbara, CA). The digitalized signals were analyzed using the computer software Biopac Student Lab Pro v.3.6.5 (Biopac Systems, Inc., Santa Barbara, CA) to obtain AP, mean arterial pressure (MAP) and HR simultaneously. The MAP was calculated as in the equation, MAP = diastolic

pressure + (systolic pressure − diastolic pressure)/3. In one group of the prepared animals, the right femoral vein was cannulated with a PE-50 tube for intravenous (I.V.) bolus administration of rat CART 61-102 or CART 55-102 peptide. Other groups of the prepared animals were administered with the CART peptides via the I.C. or intrathecal (I.T.) route.

2.4. Intracisternal administration of CART peptides

I.C. administrations were carried out in a technique previously applied (Chen et al., 2000). The rat head was fixed onto a stereotaxic device (David Kopf Instruments, Tujunga, CA). The dura above the cisternal magna of the fourth ventricle was carefully exposed by surgery without excessive bleeding. A 25-gauge needle was connected to one end of a PE-20 tube with the other end of the tube connected to a micro-syringe. The blunt-ended 25-gauge needle tip was inserted through the dura into the cisternal magna and therefore the leakage of cerebrospinal fluid is minimized.

Rat CART 61-102 and rat CART 55-102 were dissolved in artificial cerebrospinal fluid (aCSF) from Harvard Apparatus (Holliston, MA) and infused into the cisternal magna in a fixed volume of 5 μl over 1 min. Experiments with I.C. injection of 5 μl aCSF were carried out in the rats of the control group. One I.C. injection was made to each animal.

2.5. Intrathecal administration of CART peptides

Adult male Sprague–Dawley rats were prepared for measurements of AP and HR and the dura above the cisternal magna of the 4th ventricle was exposed. Similar to a previously applied technique (Lai et al., 2000), the dura was carefully cut to make a 1-mm wide opening. With one end connected to a Hamilton syringe, the other end of a PE-10 tube was passed through the opening on the dura at the atlanto-occipital junction into T2–T3 segments; the position of the tubing was visually verified at the end of the experiments. CART peptides were dissolved in aCSF and delivered intrathecally by the PE-10 tubing with the syringe at a volume of 10 μl. No more than one injection was made to any individual animals.

2.6. Immunohistochemistry

Two hours after I.C. administered with CART 61-102, the rats were transcardially perfused with cold 0.1 M phosphate buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M PBS. The brain stem was removed, postfixed in the same fixative for 2 h and then immersed in 30% sucrose/PBS for 3 days. Brain stem containing areas of interest was coronally sectioned into 40 μm with a cryostat (Cryotome SME, Shandon). Free-floating brainstem sections were processed for c-Fos-like immunoreactivity using the avidin-biotin-peroxidase complex method. Briefly, the sections were treated with 3% H_2O_2, washed, blocked with 10% normal goat serum and then incubated with rabbit polyclonal c-Fos antiserum (1:400 diluted in PBS containing 0.4% Triton X-100 and 1% bovine serum albumin) for 24 h in room temperature followed by 24 h at 4 °C with gentle agitation. After several washes with PBS, the sections were incubated with biotinylated goat anti-rabbit IgG (1:100, Vector Laboratories) for 2 h followed by avidin–biotinylated peroxidase complex (1:100, Vector Laboratories) for 1 h at room temperature. After thorough rinsing in Tris-buffered saline, sections were developed with a 3,3′-diaminobenzidine (DAB) substrate kit (Vector Laboratories). Blank control experiments were performed using antiserum preadsorped with an excess of antigen or omission of the primary antiserum.

c-Fos-positive cells of bilateral nuclei were examined and counted manually using a up-right microscope (Microphot-FXA, Nikon). Anatomical localization of the RVLM was defined according to the atlases in "The Rat Brain in Stereotaxic Coordinates" (Paxinos and Watson, 1997). Accordingly, counts were performed on every section from the level rostral to area postrema to the level caudal to facial nucleus and numbers of neurons were obtained from individual animals as averages of c-Fos-positive cells per 40-m section bilaterally. Only cells with a discernable nucleus were counted as c-Fos-positive cells. Group averages of c-Fos-positive cell numbers per section were then calculated from those of five CART 61-102 treated rats and five vehicle control rats.

2.7. Data analysis

Data were expressed as means ± standard error of the mean (SEM). Significant differences between treatment groups and dose- and time-dependent relationships were determined using one-factor or two-factor analysis of variance (ANOVA) followed by multiple comparisons using the Student–Newman–Keuls test at $p = 0.05$.

3. Results

3.1. Effects of intracisternal CART on AP and HR

CART 61-102 and CART 55-102 administered I.C. increased AP and HR in urethane-anesthetized rats. Representative recorded tracings of the increased AP and HR in rats treated with I.C. CART 61-102 (4 nmol) or I.C. CART 55-102 (4 nmol) were presented in Fig. 1. Changes in AP and HR after an I.C. injection of CART peptide at different doses to individual rats were normalized to their basal AP and HR before the I.C.

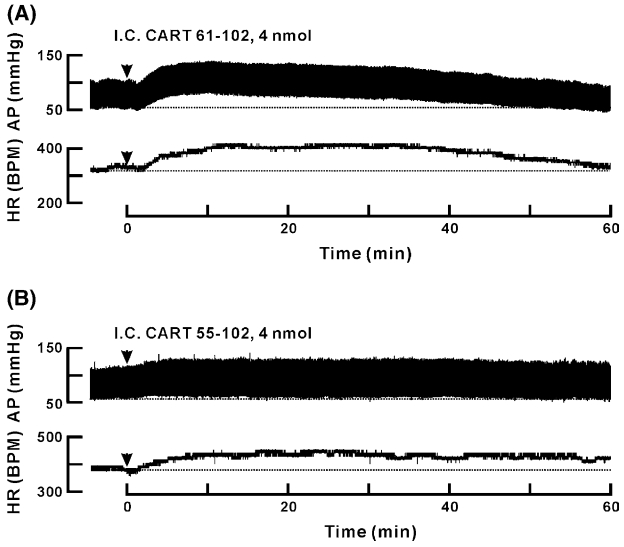

Fig. 1. Recorded tracings of AP and HR by intracisternal CARTs. Representative responses of CART 55-102 (4 nmol) and CART 61-102 (4 nmol) on the cardiovascular functions after administered into the cisternae magna in urethane-anesthetized rats.

treatment and summarized in Fig. 2. After an I.C. injection of CART peptide, the increased AP gradually returned toward the baseline at 40–60 min after the I.C. injection, at which time the HR remained elevated in several cases (Fig. 2).

Fig. 2(A) shows dose- and time-dependent changes of MAP and HR following I.C. CART 61-102 treatments. The pressor effect of I.C. CART 61-102 in MAP developed earlier than that in HR. Onset of the effect was within 2 min for MAP and was within 5 min for HR, respectively, after I.C. CART 61-102 of 2 and 4 nmol. After an I.C. injection of CART 61-102, the maximal increases of MAP and HR were attained at approximately 6–8 and 100–15 min, respectively.

On the other hand, I.C. CART 55-102 of 2 and of 4 nmol also caused an increase in MAP and HR (Fig. 2(B)). However, the pressor effects of I.C. CART 55-102 were less potent than those of I.C. CART 61-102 at the same dose. The duration of increase in HR produced by I.C. CART 55-102 was longer lasting as compared to that of MAP (Fig. 2(B)), which was also observed in I.C. CART 61-102. I.C. injection of the vehicle aCSF of equal volume caused no significant changes in MAP and HR in the control group. The basal MAP and HR of different groups of rats before I.C. treatments were summarized in Table 1 and showed no significant difference ($p > 0.1$, ANOVA).

3.2. Effects of intrathecal and intravenous injections of CART on AP and HR

CART 61-102 and CART 55-102 of 4 nmol each injected I.V. ($n = 3$ for each peptide) or I.T. ($n = 4$ and 6, respectively) caused no significant changes of MAP and HR. Representative tracings for AP and HR after I.V.

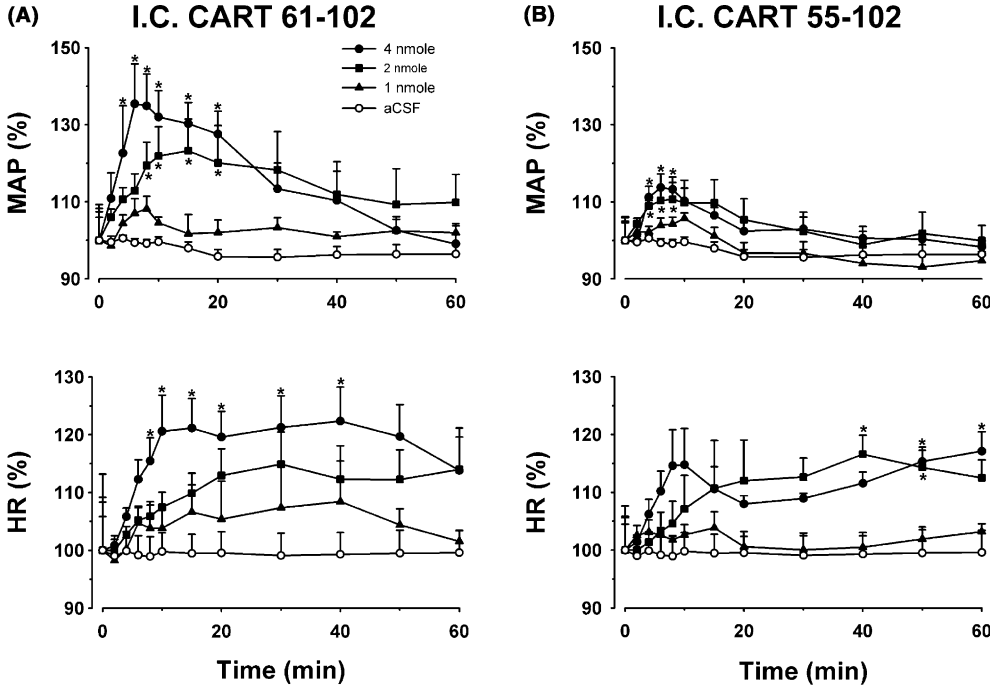

Fig. 2. Changes of MAP and HR by intracisternal CARTs in urethane-anesthetized rats. Intracisternal CART 55-102 and CART 61-102 of various doses induced dose-dependent increases in MAP and HR, respectively ($p < 0.01$, ANOVA). Symbols in (A): ▲, 1; ■, 2; and, ●, 4 nmol CART 61-102. Symbols in (B): ▲, 1; ■, 2; and ●, 4 nmol CART 55-102. The aCSF vehicle control (○) was also performed. Data were expressed as means ± SEM. (*) Significantly different from aCSF control by ANOVA followed by multiple comparisons with Student–Newman–Keuls test at $p = 0.05$.

Table 1
Basal MAP and HR in rats of different treatment groups

Treatment	No. of Animals	Basal MAP (mmHg)	Basal HR (beats/min)
aCSF	11	89 ± 5	341 ± 20
CART 61-102			
1 nmol	3	88 ± 6	353 ± 46
2 nmol	6	89 ± 7	318 ± 29
4 nmol	5	85 ± 6	307 ± 26
CART 55-102			
1 nmol	4	81 ± 4	311 ± 24
2 nmol	6	85 ± 5	321 ± 18
4 nmol	7	91 ± 4	367 ± 17

Basal mean arterial pressure (MAP) and heart rate (HR) in different treatment groups were expressed as mean ± standard error of mean (SEM). CART peptides were dissolved in artificial cerebrospinal fluid (aCSF) and intracisternally administrated in a fixed volume of 5 μl to each animal. aCSF was used as the vehicle control. No significant difference in basal MAP and HR was observed among groups ($p > 0.1$, ANOVA).

and I.T. CART 61-102 were shown in Figs. 3(A) and (B), respectively.

3.3. Intracisternal CART increased c-Fos-like immunoreactivity in RVLM

Since CART 61-102 exhibited higher central cardiovascular pressor effect than CART 55-102, the brainstems obtained from rats treated with I.C. CART 61-102 of 4 nmol were further examined for c-Fos-like immunoreactivity. By comparing to the vehicle, I.C. CART 61-102 of 4 nmol increased c-Fos-like immunoreactivity in the RVLM area (Fig. 4). The number of c-Fos positive cells per 40 μm tissue thickness in the RVLM is 100 ± 12 ($n = 5$) in I.C. CART 61-102-treated animals and is significantly higher ($p < 0.05$) than 64 ± 12 ($n = 5$) in non-treated vehicle control rats. Staining of c-Fos was absent in all immunohistochemical control experiments.

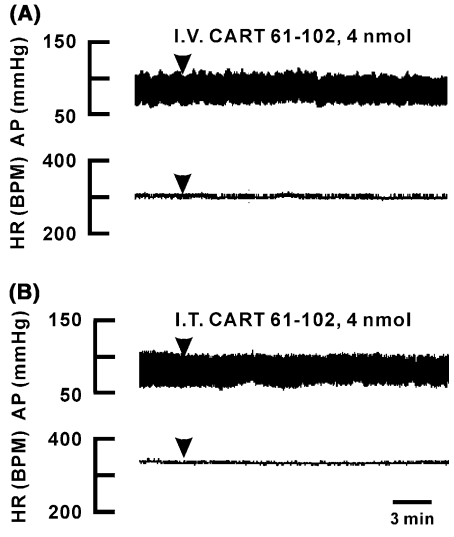

Fig. 3. Effects of intravenous and intrathecal CARTs on AP and HR. Intravenous CART 61-102 (A) and intrathecal CART 61-102 (B) did not show significant changes on blood pressure and heart rate in urethane-anesthetized rats.

4. Discussion

In summary, we observed that the two CART peptides, CART 61-102 and CART 55-102, dose-dependently caused a significant increase of AP and HR after I.C. administered to urethane-anesthetized rats whereas no significant changes in AP and HR were observed following I.V. or I.T. administration of the peptides suggesting the brain stem as a potential target structure of the two CART peptides. I.C. CART 61-102 induced greater effects in increasing MAP and HR than I.C. CART 55-102 did at same doses. Additionally, we detected an increase of c-Fos-like immunoreactivity in the rat RVLM following an I.C. CART 61-102 indicating the involvement of RVLM in the central pressor effects of CART peptides. Several biological activities of CART peptide fragments have been reported (reviewed by Kuhar et al., 2002). Here, we report central cardiovascular pressor effects of I.C. CART 61-102 and CART 55-102 in rats and the pressor effects of CART 61-102 were examined, for the first time, and compared with that of CART 55-102.

CART-immunoreactive fibers were found in the brainstem nuclei known to be associated with the regulation of cardiovascular functions, such as the nucleus of the solitary tract (NTS), area postrema, RVLM, ambiguus nuclei and raphe nuclei (Koylu et al., 1998), implying the brainstem as an important area relaying CART peptides-regulated functions. In the present study, CART peptides were delivered intracisternally into the fourth ventricle to produce a local high concentration of the peptides in the CSF circulating around the rat brainstem. Since I.T. CART 61-102 or CART 55-102 did not cause significant changes in cardiovascular functions, the brainstem appears to be the major site of action responsible for the cardiovascular effects of I.C. CART 61-102 and CART 55-102. Consistently, a previous study also suggested that the inhibitory effect of I.C.V. CART 55-102 on food intake was mainly mediated via a hindbrain site (Aja et al., 2001). The brainstem nuclei, NTS, RVLM, and caudal ventrolateral medulla (CVLM) are the primary components of the baroreceptor reflex circuitry underlying central cardiovascular regulation (Guyenet et al., 1996). Other medullary sites such as the parabrachial nucleus and area postrema are also believed to be involved in the central regulation of cardiovascular function. These brainstem nuclei could potentially be the targets of I.C. CART peptides and further studies are needed to elucidate these issues.

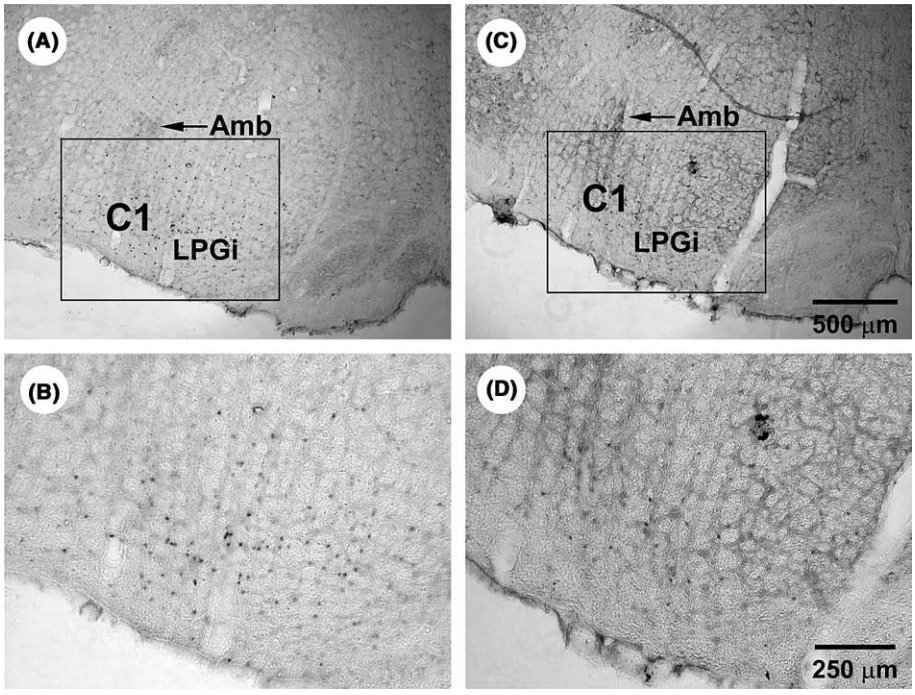

Fig. 4. Increased c-Fos-positive RVLM neurons after intracisternal administration of CART. (A) Low magnification of a section harvested from a rat treated with I.C. CART 61-102 showing the distribution of c-Fos-positive neurons in rostral ventromedulla (C1 and LPGi area). Amb, nucleus ambiguous; C1, C1 adrenergic neurons; LPGi, lateral paragigantocellular nucleusetsuedu. (B) Higher magnification of an area outlined in (A). (C) Low magnification of a section harvested from a non-treated rat. (D) Higher magnification of an area outlined in (C).

In the present study, detection of an increase of c-Fos-like immunoreactivity in the rat RVLM following an I.C. CART 61-102 indicates the involvement of RVLM in the central pressor effects of CART peptides. The RVLM is believed to be a critical pressor area where some of the neurons project directly to sympathetic preganglionic neurons in the spinal cord (reviewed by Dampney, 1994). This result suggests that the two CART peptides are capable of regulating brainstem cardiovascular sympathetic outflow. Consistently, Matsumura et al. (2001) reported that I.C.V. CART 55-102 increased AP, HR, renal sympathetic nerve activity, and plasma concentrations of epinephrine and norepinephrine in conscious rabbits suggesting activation of the sympathetic nervous system as an important mechanism for the cardiovascular effects of the peptide I.C.V. applied. A recent study reported a significant increase in the number of c-Fos-like immunoreactive neurons in central baroreceptor pathway, including NTS, CVLM and RVLM, in dog with obesity hypertension indicating a sustained activation of the central baroreceptor pathway in obesity-related hypertension (Lohmeier et al., 2003). The involvement of RVLM in the pressor effects of I.C. CART peptides is, therefore, rational and its activation provides a potential mechanism underlying obesity-related hypertension. However, the increased number of RVLM neurons with c-Fos expression does not necessarily indicate a direct activation on RVLM neurons by I.C. CART peptides. The positive c-Fos staining of RVLM neurons could be due to direct and/or indirect activations of the neurons by I.C. CART peptides. Therefore, how CART peptides affect the neuronal activity of RVLM neurons will need to be further investigated.

RVLM area was found to contain CART peptides-immunoreactive neurons (Koylu et al., 1998), which directly project to sympathetic preganglionic neurons of the intermediolateral cell column (IML) in the thoracolumbar spinal cord (Dun et al., 2002). In the present study, lack of significant changes of AP and HR upon I.T. CART peptides at the T2–T3 levels suggests that IML is not a major action site of CART peptides to produce cardiovascular effects.

Expression of CART in the hypothalamus appears to be closely regulated by leptin (Kristensen et al., 1998). Leptin is a hormone secreted by the adipocytes and its serum concentration reflects body adiposity. Higher plasma levels of leptin were detected in obese human (Considine et al., 1996) and evidences support a pressor effect of leptin (Ogawa et al., 1998; Shek et al., 1998) implying a key role of leptin in obesity-related hypertension. Evidences suggest that the expression of CART is up-regulated by leptin. For example, Kristensen et al. (1998) first reported that levels of CART mRNA were reduced in arcuate nucleus, dorsomedial and lateral hypothalamic area of the leptin-deficient ob/ob mouse and of the leptin-receptor-deficient Zucker rat, and intraperitoneal administration of recombinant leptin into

ob/ob mice caused an elevation of CART-mRNA levels in the arcuate nucleus and lateral hypothalamus. Furthermore, Elias et al. (1998) showed that CART peptide(s) were found in some hypothalamic cells (medial parvocellular part of the paraventricular nucleus, posterior part of the dorsomedial nucleus, supraoptic and arcuate nucleus) whose c-Fos levels were increased by leptin injections. The pressor effect of CART observed in the present study leads to a hypothesis that CART is one of the underlying signaling pathways responsible for the pressor effects of leptin. Consistently, both leptin and CART exert their pressor effects with mechanisms closely linked to autonomic nervous system in the present and previous studies (Haynes et al., 1997; Matsumura et al., 2001). The study on the effects of CART peptides on activities of neurons pertaining to the regulation of cardiovascular functions is warranted.

Matsumura et al. (2001) have reported that I.C.V. CART 55-102 increased AP at 0.1 or 1 nmol and elevated HR at 1 nmol in conscious rabbits and the effects lasted for 90–120 min. In the present study, two CART peptide fragments, CART 55-102 and CART 61-102 I.C, were examined and both CART fragments exhibited cardiovascular pressor effects after I.C. administered to urethane-anesthetized rats. CART 61-102 appears to be more effective than CART 55-102. Higher doses, 1–4 nmol, of CART 55-102 were required to produce cardiovascular effects. This might be due to different species of animals, different sites of administration in two studies and/or to the influence of anesthetics used in the present study. On the other hand, CART 55-102 administered I.C.V. at 1 or 2 μg (i.e. 0.19 or 0.38 nmol) elicited anorectic effect in non-fasted and neuropeptide Y-challenged rats (Kristensen et al., 1998). However, at these lower doses, CART 61-102 and CART 55-102 did not cause significant changes in AP and HR in the present study.

The activity of CART 61-102 is more potent than CART 55-102 in the central cardiovascular control while applied I.C. and may also be potentially more potent than CART 55-102 on the other pharmacological effects observed with CART 55-102. Differential effects of I.C.V. CART 61-102 and I.C.V. CART 55-102 on inhibiting the feeding behaviors have been previously observed and inconsistent results were reported. Thim et al. (1998) reported CART 55-102 appeared to be more potent in suppressing food consumption than CART 61-102, CART 62-102, and CART 54-102 in female NMRI mice, whereas Bannon et al. (2001) observed that the minimally effective dose (0.1 μg) of CART 61-102 (i.e. CART 49-89 of rat short form pro-CART peptide) in reducing food intake was 5-fold less than that (0.5 μg) of CART 55-102 (i.e. CART 42-89 of rat short form pro-CART peptide) in male mice. It is, however, not known whether mice of different sexes of the same strain respond differently to the CART peptides.

Differential activity profiles among different CART peptide fragments have been observed. In the present study, the minimally effective dose of I.C. CART 55-102 in cardiovascular effects was 2 nmol, which is 5- to 20-fold higher than the doses of CART 55-102 active on other biological effects in rats. These effects include an inhibition of food intake by I.C.V. (Kristensen et al., 1998) or I.C. CART 55-102 (Okumura et al., 2000), an increase in circulating corticosterone, oxytocin and glucose by I.C.V. CART 55-102 (Vrang et al., 2000), and a suppression of gastric acid secretion and gastric emptying by I.C. CART 55-102 (Okumura et al., 2000). What might be the physiological and/or pathological significance of CART peptides to exert their cardiovascular effects at a higher dose? As leptin is capable of up-regulating the expression level of CART, the level of released CART peptides may be elevated in obese subjects. Therefore, the impact of regulating cardiovascular functions by the CART peptides may be greater in obese subjects. Furthermore, since CART 61-102 is more potent than CART 55-102 in the central pressor effects, CART 61-102 may be more important than CART 55-102 in the regulation of certain physiological, besides the cardiovascular, functions. Nevertheless, whether different CART peptide fragments exert differential effects on different functions via acting on various brain nuclei is to be further investigated.

Acknowledgements

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