Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 494 (2004) 179–182

www.elsevier.com/locate/ejphar

Short communication

CART peptide increases the mesolimbic dopaminergic neuronal activity: a microdialysis study

Shu-Chuan Yang[a], Jenn-Tser Pan[a], Hui-Yun Li[b,*]

[a] Department of Physiology, School of Medicine, National Yang-Ming University, Taipei 112, Taiwan, ROC
[b] Department of Anatomy, School of Medicine, Chang Gung University, Tao-Yuan 333, Taiwan, ROC

Received 18 March 2004; accepted 12 May 2004

Abstract

The effects of cocaine-and amphetamine-regulated transcript (CART) peptide on extracellular concentrations of dopamine metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the shell region of the nucleus accumbens (AcbSh) were determined by microdialysis in conscious rats. Intracerebroventricular injections of various doses (0.1–5 μg/5 μl/rat) of $CART_{55-102}$ elicited dose-dependent increases of extracellular DOPAC and HVA concentration in the AcbSh, suggesting that $CART_{55-102}$ peptide has a psychostimulant-like effect via activation of the mesolimbic dopaminergic system.

Keywords: Nucleus accumben; 3,4-Dihydroxyphenylacetic acid; Homovanillic acid; Ventral tegmental area

1. Introduction

Cocaine- and amphetamine-regulated transcript (CART) mRNA was first identified in rat striatum after acute administration of cocaine or amphetamine (Douglass et al., 1995), suggesting CART peptide might be related to the mechanisms underlying the actions of psychomotor stimulants (Kuhar and Dall Vechia, 1999). Anatomical studies have found that CART-immunoreactive nerve terminals have a widespread distribution in rat brain, including the ventral tegmental area and nucleus accumbens (Koylu et al., 1998; Smith et al., 1999). Recent studies further demonstrated that intra-ventral tegmental area injection of CART peptide significantly increases locomotor activity and promotes conditioned place preference in rats (Kimmel et al., 2000), indicating these peptides are likely to be modulators of mesolimbic dopaminergic system.

The nucleus accumbens is one of the major projection sites of mesolimbic dopaminergic neurons originating from the ventral tegmental area (Cooper et al., 2003). Central infusion of cocaine or amphetamine has been shown to increase dopamine release in the nucleus accumbens and enhance locomotor activity in rats (Andrews and Lucku, 2001; Heidbreder et al., 1999). The increase of extracellular dopamine concentration in the nucleus accumbens is believed to be one of the main mechanisms involved in the rewarding and motor-activating properties of psychostimulants (Di Chira, 1995; Pontieri et al., 1995; Wise and Bozarth, 1987).

In view of the above findings, a functional interplay between CART peptide and mesolimbic dopaminergic neurons may be similar to the mechanisms by which psychomotor stimulants exert their effects on motor activity. To further validate the effect of CART peptide on dopaminergic activity in the nucleus accumbens, microdialysis was used to detect the levels of dopamine metabolites after intracerebroventricular (i.c.v.) injection of CART peptide in freely moving rats.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, weighing 220–290 g, were purchased from the National Experimental Animal Breeding

* Corresponding author. Tel.: +886-3-2118800x5447; fax: +886-3-2118112.
E-mail address: hyli@mail.cgu.edu.tw (H.-Y. Li).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.05.018

and Research Center of the National Science Council (Taipei, Taiwan). Animals were housed individually in a controlled environment with 14–10 h light–dark cycle and allowed free access to food and water. The experimental procedures were carried out in accordance with the European Community guidelines for the use of experimental animals and approved by the Institutional Animal Care and Use Committee of Chang Gung University on Animal Research.

2.2. Surgical implantations of microdialysis probes

Each rat was implanted with two guide cannulae for microdialysis into the shell region of the left nucleus accumbens (AcbSh) (AP: +1.3 mm, ML: −0.8 mm, DV: −6.2 mm) and i.c.v. injection into the right lateral ventricle (AP: 0, ML: +1.4 mm, DV: −3.2 mm), respectively (Paxinos and Watson, 1997). The homemade concentric microdialysis probes were constructed with an active membrane (Spectra/Pro RC 132280, internal diameter 0.2 mm, 13000 molecular weight cutoff, Spectrum Laboratories, CA, USA) approximately 1 mm in length.

2.3. In vivo microdialysis

All microdialysis experiments were performed in freely moving rats in their home cages with food and water available. On the day of the experiment, the dummy cannula aimed at the AcbSh was removed and replaced immediately with a homemade dialysis probe. The probe was connected to a microdialysis pump (CMA/102, CMA Microdialysis, North Chelmsford, MA) and perfused with artificial cerebral spinal fluid (aCSF: NaCl, 122.9 mM; KCl, 4.8 mM; $CaCl_2$, 1.2 mM; $MgSO_4$, 1.2 mM; and Na_2HPO_4, 12 mM, pH 7.4) at a flow rate of 1 μl/min. After 60 min of equilibration, dialysis samples were collected every 20 min and the first three samples were used as baseline. Animals were then infused with aCSF ($n=5$) or different doses (0.1, 0.5, 1 or 5 μg/5μl in 5 min, $n=6$ for each dose) of $CART_{55-102}$ (American Peptide, Sunnyvale, CA) through the i.c.v. cannulae. The perfusate was collected every 20 min for 280 min, acidified with perchloric acid, and stored immediately at −80 °C until analyzed.

2.4. High-performance liquid chromatography

The concentrations of the dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined with high performance liquid chromatography (HPLC) coupled with electrochemical detection (ECD) as reported previously (Shieh and Pan, 1999). In brief, the dialysates (10 μl/sample) was injected into an HPLC-ECD system (BAS LC480, with PM-80 pump, Rheodyne 7125 injector, phase II ODS column, 3.2 × 100 mm with 3-μm spheres, and an LC-4C EC detector, Bioanalytical Systems, West Lafayette, IN) composed of a mobile phase consisting of 0.775 mM sodium octanesulphonate, 0.5 mM EDTA, 0.171 mM NaH_2PO_4 with 12% methanol (pH 2.8). The flow rate of the pump was 0.8 ml/min, and the oxidizing potential was set at +0.75 V. To quantify the sample peaks, each chemical was compared with the external standards which were prepared freshly and injected every five sample runs.

2.5. Histological verification of the probe placement

At the end of experiments, animals were perfused via the ascending aorta with 4% paraformaldehyde (pH 9.5), and the brains were postfixed for 4 h and cryoprotected in 20% sucrose in 0.1 M phosphate buffer (pH 7.4) overnight at 4 °C. Series of 30-μm-thick frozen coronal sections throughout the striatum were collected and processed for Nissl staining for histological confirmations of the place-

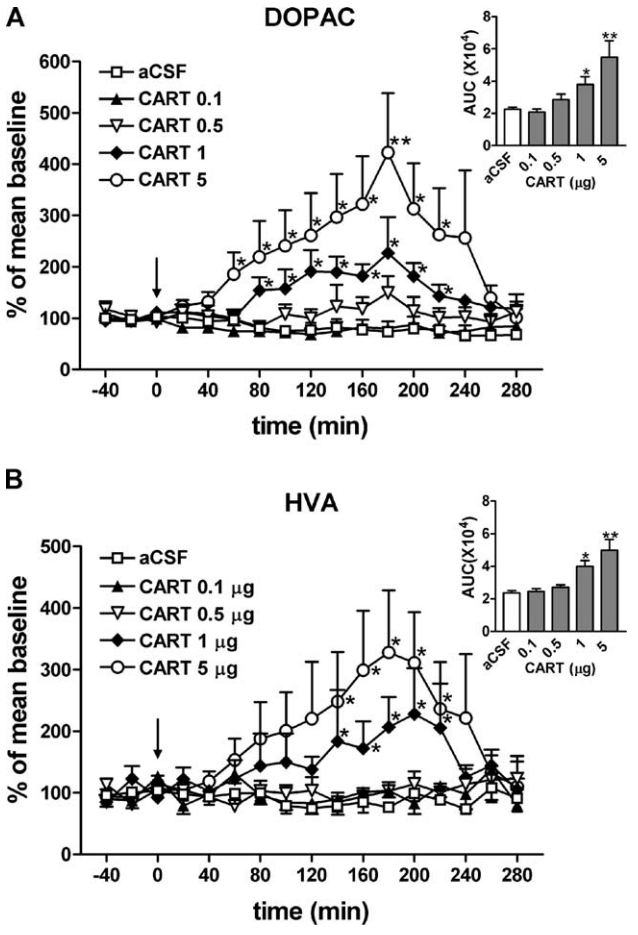

Fig. 1. Effects of i.c.v. $CART_{55-102}$ infusion on DOPAC (A) and HVA (B) levels in the AcbSh. Fractions were collected every 20 min over 320 min. Arrows indicate the time when CART peptides were infused. The data are presented as the mean percentage change from the baseline ± S.E.M.. *$P<0.05$, **$P<0.01$, significantly different from the basal value before injections. Insets demonstrate effect of i.c.v. $CART_{55-102}$ infusion on the area under the curve (AUC) for DOPAC (A) and HVA (B) levels in the AcbSh. The data are presented as the mean ± SEM. *$P<0.05$, **$P<0.01$, significantly different from animals receiving aCSF.

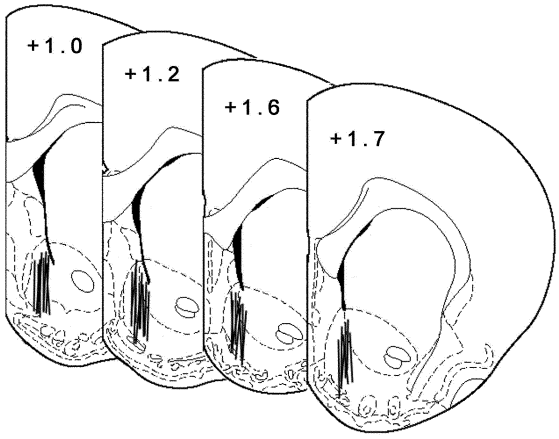

Fig. 2. Anatomical localizations of the "active" zone of microdialysis probes in the AcbSh according to the anatomical analysis of a series of consecutive brain sections bearing the trace of the microdialysis probe ($n=29$). The numbers indicate distance (mm) from bregma.

ments of cannulae. The data from rats with misplaced cannulae were not included in the analysis.

2.6. Data analysis

The DOPAC and HVA levels following CART peptide treatments are presented as the mean percentage change from the baseline. A one-way analysis of variance followed by a Newman–Keuls post hoc analysis was used to compare values before and after CART peptide treatments. A P value less than 0.05 defined statistical significance.

3. Results

The average basal values of dopamine in dialysates of the AcbSh for the different experiments did not differ significantly. Therefore, they were groups here together. Basal values ± S.E.M. for DOPAC and HVA were 1.19 ± 0.19 and 0.72 ± 0.19 pM/min, respectively ($n=87$).

As depicted in Fig. 1A, the temporal pattern of DOPAC increased significantly in rats receiving 1 μg of CART (154 ± 25%, $P<0.05$) at 80 min after injection and in rats receiving 5 μg of CART (185 ± 43%, $P<0.05$) at 60 min after injection. The level of DOPAC peaked at 180 min in animals receiving either 1 μg of CART (227 ± 70%, $P<0.05$) or 5 μg of CART (422 ± 115%, $P<0.01$), and declined gradually to basal levels at 240 min after injections. The lower dose of CART (0.5 μg) elicited a slight, but not significant, increase of DOPAC levels.

Similarly, the HVA levels in rats receiving 1 or 5 μg of $CART_{55-102}$ were elevated gradually after injections and achieved statistical significance at around 140 min after injection (183 ± 83% or 249 ± 81%, respectively, $P<0.05$), peaked at 200 min (1 μg, 228 ± 75%, $P<0.05$) and 180 min (5 μg, 328 ± 100%, $P<0.05$), and then declined to basal levels at 240 min after injections (Fig. 1B). There was no significant effect of the lower doses of CART on the extracellular HVA concentration.

To examine the effects of different doses of CART peptide on the levels of extracellular of DOPAC and HVA during the period of microdialysis, the area under the curve (AUC) was calculated (insets of Fig. 1). Notably, i.c.v. infusions of CART peptide at the doses of 1 μg ($P<0.05$) and 5 μg ($P<0.01$), significantly increased extracellular DOPAC and HVA levels.

Fig. 2 shows the graphical representations of the placement of the microdialysis membrane regions in the brain of 29 rats in this experiment. Anatomical tracing of the placement of the "active" zone of the microdialysis probes revealed that the target region was an area in the mid-region of the AcbSh.

4. Discussion

The objective of the present study was to assess the effect of central injection of various doses of CART peptides on the levels of dopamine metabolites in the AcbSh in freely moving rats. We have demonstrated that 1 and 5 μg of CART significantly elevated DOPAC and HVA release from the AcbSh in dose-and time-dependent manners. Taking into account that the increases of extracellular dopamine levels in the nucleus accumbens is associated with drug addiction and locomotor activity (Hajnal and Norgren, 2002; Zapata et al., 2003), our findings suggest that CART peptide may serve a role as a psychostimulant via facilitating the mesolimbic dopaminergic system.

The profound effect of CART peptide on elevating DOPAC levels persisted from 60 to 220 min after 5μg of CART peptide, and a similar pattern, but to a lesser extent, was observed in animals receiving 1 μg of CART peptide. Consistently, changes in extracellular HVA levels displayed a similar profile as seen for the DOPAC levels after injections of different doses of CART.

It is known that cocaine or amphetamine treatment selectively increased dopamine release in the AcbSh (Heidbreder et al., 1999). In the present study, the active zone of each microdialysis probe extended dorsoventrally through the AcbSh as shown in Fig. 2. Thus, our data demonstrate a representative profile of dopaminergic activity within this region. Extracellular DOPAC is thought to reflect changes in the intracellular pool of dopamine (Zetterstrom et al., 1986), and the increased extracellular DOPAC and HVA levels could theoretically imply a variety of factors such as increase in dopamine synthesis, decrease of active uptake process, or simply an increase in dopamine release.

Our findings that i.c.v. injection of $CART_{55-102}$ increased DOPAC and HVA levels provide mechanisms underlying previous studies that intra-ventral tegmental area, but not intra-substantia nigra injection of CART peptide facilitates locomotor activity and this stimulatory effect is blocked by dopamine D2 receptor antagonist

(Kimmel et al., 2000). It is noteworthy that anatomical evidence demonstrated that dopaminergic neurons located in the ventral tegmental area, rather than the substantia nigra, project to the AcbSh (Oades and Halliday, 1987), and CART-immunoreactive terminals were abundant in the ventral tegmental area in rats (Koylu et al., 1998) and monkeys (Smith et al., 1999). These findings strongly suggest that CART peptide exerts its function in mediating locomotor activity via regulating the mesolimbic dopaminergic system. On the other hand, in addition to be found in the dopaminergic neurons in the ventral tegmental area, CART immunoreactive terminals were also found in the AcbSh, the major mesolimbic dopaminergic terminal fields (Oades and Halliday, 1987), implying that CART peptide may interact with dopamine release in this region (Smith et al., 1999). So far, identification of CART receptors has not been achieved, and it appears that it does not interact with most of the known neurotransmitters based on binding studies (Jaworski et al., 2003). Thus, it is likely that specific receptors for CART exist and have yet to be discovered.

In summary, our findings are the first to demonstrate that the psychomotor stimulant-like effects of CART peptides are associated with increased dopaminergic activity in the AcbSh and thus provide direct evidence that CART peptides are involved in regulation of mesolimbic dopaminergic system.

Acknowledgements

We thank Drs. Jin-Chung Chen and Kun-Ruey Shieh for their helpful comments during the preparation of this manuscript. This study was supported by a grant (NSC 90-2314-B-182-085) from the National Science Council and Chang Gung Memorial Hospital, Taiwan, R.O.C. (to H.Y. Li).

References

Andrews, C.M., Lucku, I., 2001. Effects of cocaine on extracellular dopamine and serotonin levels in the nucleus accumbens. Psychopharmacology 155, 221–229.

Cooper, J.R., Bloom, F.E., Roth, R.H., 2003. Biochemical Basis of Neuropharmacology, eighth ed. Oxford Univ. Press, New York, NY.

Di Chira, G., 1995. The role of dopamine in drug abuse viewed from the perspective of its role in motivation. Drug Alcohol Depend. 38, 95–137.

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

Hajnal, A., Norgren, R., 2002. Repeated access to sucrose augments dopamine turnover in the nucleus accumbens. NeuroReport 13, 2213–2216.

Heidbreder, C.A., Hedou, G., Feldon, J., 1999. Behavioral neurochemistry reveals a new functional dichotomy in the shell subregion of the nucleus accumbens. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 23, 99–132.

Jaworski, J.N., Vicentic, A., Hunter, R.G., Kimmel, H.L., Kuhar, M.J., 2003. CART peptides are modulators of mesolimbic dopamine and psychostimulants. Life Sci. 73, 741–747.

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., Kuhar, M.J., 1998. Cocaine- and amphetamine-regulated transcript peptide immunohistochemical localization in the rat brain. J. Comp. Neurol. 391, 115–132.

Kuhar, M.J., Dall Vechia, S.E., 1999. CART peptides: novel addiction-and feeding-related neuropeptides. Trends Neurosci. 22, 316–320.

Oades, R.D., Halliday, G.M., 1987. Ventral tegmental (A10) system: neurobiology: 1. Anatomy and connectivity. Brain Res. 434, 117–165.

Paxinos, G., Watson, C., 1997. The Rat Brain in Stereotaxic Coordinates Academic Press, San Diego, CA.

Pontieri, F.E., Tanda, G., Di Chiara, G., 1995. Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens. Proc. Natl. Acad. Sci. U. S. A. 92, 12304–12308.

Shieh, K.R., Pan, J.T., 1999. Stimulatory role of prolactin on the development of tuberoinfundibular dopaminergic neurons in prepubertal female rats: studies with cysteamine and somatostatin. J. Neuroendocrinol. 11, 907–917.

Smith, Y., Kieval, J., Couceyro, P.R., Kuhar, M.J., 1999. CART peptide-immunoreactive neurons in the nucleus accumbens in monkeys: ultrastructural analysis, colocalization studies, and synaptic interactions with dopaminergic afferents. J. Comp. Neurol. 407, 491–511.

Wise, R.A., Bozarth, M.A., 1987. A psychomotor stimulant theory of addiction. Psychol. Rev. 94, 469–492.

Zapata, A., Chefer, V.I., Ator, R., Shippenberg, T.S., Rocha, B.A., 2003. Behavioural sensitization and enhanced dopamine response in the nucleus accumbens after intravenous cocaine self-administration in mice. Eur. J. Neurosci. 17, 590–596.

Zetterstrom, T., Sharp, T., Ungerstedt, U., 1986. Further evaluation of the mechanism by which amphetamine reduces striatal dopamine metabolism: a brain dialysis study. Eur. J. Pharmacol. 132, 1–9.