≪Research note≫

Intracerebroventricular Injection of Corticotropin-Releasing Factor Does not Alter Monoamine Content of the Paraventricular Nucleus of the Hypothalamus in Neonatal Chicks

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Intracerebroventricular (i.c.v.) injection of corticotropin-releasing factor (CRF) on monoamine contents of the paraventricular nucleus of the hypothalamus (PVN) was investigated in neonatal chicks. The PVN was isolated from the brains of neonatal chicks 15 minutes after the i.c.v. administration of 0, 0.01, 0.1 or 1 μg of CRF. The concentrations of noradrenaline, adrenaline, dihydroxyphenylacetic acid, dopamine, 5-hydroxyindole-3-acetic, homovanillic acid and 5-hydroxytryptamine were determined. The monoamines and their metabolites showed some alterations, but the differences were not significant among all treatments. The results suggested that CRF did not affect the monoamine contents of the PVN in the neonatal chick.

Key words : corticotropin-releasing factor (CRF), monoamines, paraventricular nucleus of the hypothalamus (PVN), chicks

Introduction

Corticotropin-releasing factor (CRF) is a key regulator of brain excitability changes associated with stress (Ehlers *et al.*, 1983), and the catecholaminergic system has been also hypothesized to be involved in mediating behavioral constructs related to alertness, arousal and stress (Koob, 1999). The release of CRF occurs during stress, and the catecholaminergic system is activated under similar condition (Koob, 1999).

Intracerebroventricular (i.c.v.) injection of CRF suppressed food intake in the neonatal chick in a dose-dependent manner (Furuse *et al.*, 1997 ; Zhang *et al.*, 2001 a). It has also been reported that noradrenaline (NA) and adrenaline (A) are involved in neural regulation of food intake in the paraventricular nucleus of the hypothalamus

(PVN) in chicks (Denbow *et al.,* 1981 ; Denbow and Sheppard, 1993).

Anatomic evidence exists for CRF-NA synaptic connections in the PVN of rats (Liposits *et al.,* 1987) and domestic fowl (Jozsa *et al.,* 1984 ; Knigge and Piekut, 1985). Liposits *et al.* (1986) suggested that the parvocellular division contains noradrenergic and adrenergic terminals on CRF immunoreactive cells, which play a role in catecholamineregic regulation of the pituitary-adrenocortical responses in rats.

Centrally administered CRF facilitated NA release in several brain regions including the hypothalamus, medial prefrontal cortex and locus coeruleus (LC) using intracerebral microdialysis in rats (Emoto *et al.,* 1993 ; Lavicky and Dunn, 1993 ; Smagin *et al.,* 1995). However, no report about the relationships between CRF and monoamine contents has been published in chicks. Thus, the present study was performed to identify the effect of i.c.v. administration of CRF on monoamine contents in the PVN of chicks.

Materials and Methods

1. i.c.v. injection of CRF

Day-old male broiler chicks (Cobb, Mori Hatchery,.Fukuoka, Japan) were individually housed in a windowless room at a constant temperature of 28°C. Lighting was provided continuously. The birds were given free access to a commercial starter diet (Toyohashi Feed and Mills Co. Ltd., Aichi, Japan) and water, and were maintained in accordance with recommendations of the National Research Council (1985). On experimental days, the birds were distributed into groups based on their body weight so that the average body weight was as uniform as possible for each treatment. The birds (2-day-old, 10 birds per group) were injected i.c.v. with either saline, 0.01, 0.1 or 1 μg of ovine CRF in a volume of 10 μl using a microsyringe according to the method of Davis *et al.* (1979).

Based on our previous report (Furuse *et al.,* 1997 ; Zhang *et al.,* 2001a), the same

Table. Effects of i.c.v. injection of CRF on monoamines and their metabolites (pg/mg tissue) in the chicken PVN

Monoamines	CRF			
	0 μg	0.01 μg	0.1 μg	1 μg
NA	3952 ± 373	3272 ± 637	4094 ± 713	4003 ± 406
A	244 ± 35	184 ± 51	238 ± 59	224 ± 26
5-HT	1379 ± 109	1524 ± 180	1802 ± 167	1632 ± 154
5-HIAA	223 ± 22	257 ± 43	281 ± 67	283 ± 37
5-HIAA/5-HT	0.164 ± 0.016	0.175 ± 0.033	0.152 ± 0.028	0.187 ± 0.033
DA	434 ± 23	454 ± 65	767 ± 187	564 ± 93
HVA	147 ± 12	167 ± 17	195 ± 34	185 ± 19
HVA/DA	0.342 ± 0.027	0.386 ± 0.036	0.281 ± 0.023	0.375 ± 0.059
DOPAC	31 ± 2	37 ± 4	66 ± 18	47 ± 5
DOPAC/DA	0.074 ± 0.006	0.084 ± 0.007	0.085 ± 0.006	0.089 ± 0.008

Values are means ± S.E.M. The number of birds used was as follows : control, 8 ; 0.01 μg, 7 ; 0.1 μg, 7 ; 1 μg, 7.

doses were applied in the present study since CRF effectively suppressed the feed intake of chicks, even in a low dose of 0.01 µg. Ovine CRF purchased from Peptide Institute Inc. (Osaka, Japan) was dissolved in a 0.1% Evans Blue solution which was prepared in 0.85% saline. Saline was used as a control.

2. Detection of monoamines of the PVN

After 15 minutes post-injection, the birds were decapitated under ether anesthesia. The brains from individuals in which the dye was confirmed to be in the lateral ventricle were removed and stored at $-80°C$. Both sides of PVN were carefully dissected with the commissura anterior as a mark, and the tissue was weighed. The samples were homogenized for 30 seconds in 80 µl 0.2 M perchloric acid (Katayama Chemical Co. Ltd., Osaka, Japan) containing isoproterenol (Sigma, Louis, MO, USA) as an internal standard. After being centrifuged (9000 x g for 4 min at 4°C), the supernatant was transferred into centrifuge-filtration filter (Ultra Free C3-GV, Millipore, Bedford, MA, USA) and centrifuged again at the same condition. The 30 µl of filtrate were assayed for NA, A, dopamine (DA), 5-hydroxytryptamine (5-HT) and their metabolites dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydro-xyindole-3-acetic acid (5-HIAA) using high performance liquid chromatography with electrochemical detection (HPLC-ECD) (HPLC, Eicom, Kyoto, Japan ; ECD-300, Kyoto, Japan) with a reverse-phase column (EICOMPAK SC-50DS, Kyoto, Japan). The mobile phase consisting of 39.8 mM sodium acetate (Sigma, Tokyo, Japan), 44.2 mM citric acid (Katayama Chemical Co. Ltd., Osaka, Japan), 190 mg/l sodium 1-octanesulfonate (Tokyo Kasei Kogyo Co. Ltd., Osaka, Japan), 5 mg/l disodium ethylenediaminetetraacetate (Katayama Chemical Co. Ltd., Osaka, Japan) 16% meth-anol (Katayama Chemical Co. Ltd., Osaka, Japan) was employed at a 0.2 ml/min rate. The results were expressed as pg/mg wet weight of PVN.

3. Statistical Analysis

The data were analyzed by one-way analysis of variance, and fitted to regression analysis using StatView (Version 5, SAS Institute, Cary, USA). The results are presented as means±S.E.M.

Results and Discussion

As shown in Table, no significant changes in monoamines, or their metabolites, were observed in the chicken PVN following the i.c.v. injection of CRF. A significant regression equation was obtained for DOPAC (DOPAC=32.3 (SE 6.6) +373.1 (SE 129.2) CRF−358.2 (SE 125.6) CRF2, df=26, p<0.05, R^2=0.245). The metabolism ratios of 5-HIAA/5-HT, HVA/DA and DOPAC/DA were calculated to clarify whether the rates of turnovers were changed. However, no significant differences were detected in these parameters.

Dunn and Berridge (1990) reported that a dose, higher than that required to elicit several behavioral effects, was required to activate the cerebral catecholaminergic system. Lavicky and Dunn (1993), using microdialysis, concluded that CRF dose-dependently stimulated catecholamine release with application of 17 and 330 pmol (i.e., 0.079 and 1.541 µg respectively) of CRF in rats. Furthermore, it was also reported that

i.c.v. injection of CRF increased NA release in the rat PVN at a dose of 3μg (Emoto *et al.,* 1993). On the contrary, Dunn and Berridge (1987) did not find any changes in 5-HT, or its metabolite 5-HIAA, after i.c.v. infusion of 1.0μg CRF. In poultry, i.c.v. injection of $5.6-30\mu$g/kg of CRF increased the concentration of 5-HIAA in pigeons (Barrett *et al.,* 1989). Taken together, all available data indicated that high levels of CRF stimulated monoamine release. The present results showed that monoamines and their metabolites tended to increase following 0.1μg of CRF, but content was not further enhanced by the 1μg dose. However, the three doses of CRF applied here have been shown to significantly modify the behavior of the neonatal chick (Furuse *et al.,* 1997 ; Zhang *et al.,* 2001a, b). This implies that a wider range of doses should be applied in chicks to clarify the relationship between CRF and catecholamines, or the mechanism of regulation of CRF in chicks is largely different from that in mammals. Furthermore, Lavicky and Dunn (1993) reported that the response of monoamines to CRF commenced within 20 min and reached a maximum at $\sim$1.5-2 h, then returned to baseline within 3 h. According to our previous report (Zhang *et al.,* 2001b), the behaviors of chicks significantly changed within 15 min after i.c.v. injection of CRF. Thus, it is believed that CRF should modify neurotransmitters within the time applied here. However, we only determined monoamine contents of the PVN, not monoamine release. Microdialysis analysis is required in future to investigate monoamine release.

It has been shown that i.c.v. administration of DA into the lateral ventricle has no effect on food intake in the chick (Denbow *et al.,* 1981). In the present study, the difference of DOPAC was nearly significant (p$=$0.06), but DA and DOPAC/DA were comparable among all doses. Further investigation will be required for elucidating the relationship between DOPAC and stress.

Wellman (2000) reported that two subtypes of α-adrenergic receptors within the PVN exerted antagonistic actions on eating in the rat. Activation of PVN α_2-adrenoceptors increased eating, whereas activation of PVN α_1-adrenoceptors suppressed eating. The results obtained in chicks showed that the food intake was suppressed by a α_2-adrenoceptor antagonist, yohimbine, but not by a α_1-adrenoceptor antagonist, prazosin (Bungo *et al.,* 2001). Plotsky (1987) reported that NA largely stimulated CRF release in the PVN through α_1-adrenoceptors in rats. Taken together, the results imply that regulation mechanisms of food intake by CRF and monoamines are different between avians and mammals.

It has been shown that CRF activates the noradrenergic neurons in the LC which contains as many as half of all NA neurons in the brain (Smagin *et al.,* 1995). Similar studies are required in the LC of chicks to further understand the regulation mechanism of stress.

In the present study, central CRF did not alter monoamine contents of the PVN in birds. This result suggests that CRF and monoamine do not work synergically for stress in birds and this different mechanism may protect birds from external noxious stimuli.

Acknowledgements

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