《Research Note》

Intracerebroventricularly Administration of Glucagon-Like Peptide-1 Induces Sleep-Like Behavior in the Neonatal Chick

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It is known that central administration of glucagon-like peptide-1 (GLP-1) (7-36) amide suppresses feeding in rats and chicks, but the mechanisms for this response are still open with special reference to the chick. The present study was done to determine whether GLP-1 induces sleep-like behavior on the neonatal chick. Intracerebroventricular injection of GLP-1 (7-36) amide altered, in a dose-dependent fashion, the behavioral postures of chicks and induced sleep-like behavior compared with the saline control. These results indicate that GLP-1 in the central nervous system may be one of the factors to induce sleeping of the neonatal chick.

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Key words : glucagon-like peptide-1 (GLP-1), sleep-like behavior, intracerebroventricular injection, neonatal chick

Introduction

Glucagon-like peptide-1 (GLP-1) is recognized as an inhibitor of food intake in rats (TURTON *et al.*, 1996 ; NAVARRO *et al.*, 1996 ; TANG-CHRISTENSEN *et al.*, 1996) and chickens (FURUSE *et al.*, 1997 a, b), but the mechanisms for this anorexigenic effect of GLP-1 are poorly defined. One of the systems suggested by LARSEN *et al.* (1997) for the central GLP-1 depression of food intake in the rats is by GLP-1 activation of the hypothalamo-

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pituitary-adrenocortical axis. This is primarily stimulation of corticotropin releasing factor neurons, and this activation may be responsible for the inhibition of feeding behavior. In fact, plasma corticosterone concentration was rapidly increased after central administration of GLP-1 (7-36) amide in the rat (WANG *et al.*, 1996). On the contrary, central GLP-1 (7-36) amide did not alter plasma corticosterone level in the neonatal chick (FURUSE *et al.*, 1997 c). Central nervous system of GLP-1 action in avian species has not been clarified.

Although there was no systematic attempt to quantify behavior, FURUSE *et al.* (1997 c) found that the chicks were very calm and moved less after intracerebroventricular (i.c.v.) injection of GLP-1. This finding hypothesized that sleep-like behavior induced by GLP-1 (7-36) amide may contribute to the anorexigenic effect of GLP-1 in the neonatal chick. Thus, it is important to quantify the narcoleptic response, which was caused by GLP-1 for the clarification of central nervous system of GLP-1 action.

To confirm the relationships between GLP-1 and sleep-like behavior, therefore, the categorized posture of neonatal chicks was observed after central administration of chicken GLP-1 (7-36) amide.

Materials and Methods

Day-old male Single Comb White Leghorn chicks (Julia ; Murata Hatchery, Fukuoka, Japan) were 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. They were distributed into experimental groups based on their body weight, so that the average body weight was as uniform as possible for each treatment. After being distributed into each group, birds (3-day-old, 10 birds per group) were observed the postures at 30 min immediately after injection, because anorexigenic effect of GLP-1 (7-36) amide (0.03 µg) became fainter after 60 min following administration (FURUSE *et al.*, 1997 b). As referring to VAN LUIJTELAAR *et al.* (1987), four behavioral categories were distinguished : (1) active wakefulness ; (2) standing/sitting with eyes open ; (3) standing motionless with eyes closed ; (4) sitting motionless with head drooped (sleeping posture). Observation was performed by a trained observer who was not informed of each treatment.

The birds were i.c.v. injected with the solutions (10 µl) using a microsyringe according to the method of DAVIS *et al.* (1979). Chicken GLP-1 (7-36) amide was purchased from Peptide Institute, Inc. (Osaka, Japan) and was dissolved in a 0.1% Evans Blue solution, which was prepared in 0.85% saline. GLP-1 (7-36) amide was administered at the dose levels of 0, 0.01, 0.03 and 0.1 µg. At the end of the experiment, the birds were sacrificed by decapitation, followed by brain sectioning to identify the location of injected drug. Data from the individuals that were not verified to have Evans Blue dye present in the lateral ventricle were deleted. The number of birds used was as follows : 9 (0 µg) ; 9 (0.01 µg) ; 8 (0.03 µg) and 8 (0.1 µg).

Data were subjected to t-test for ordered classifications (SNEDECOR and COCHRAN, 1967) between control and each dose of GLP-1 (7-36).

Table 1. Influence of GLP-1 (7-36) amide on the various behavioral categories of the neonatal chick after 30 min following administration

Treatment (n)		Number of chicks with the postures of score*				Pvs the control for posture score
		1	2	3	4	
Control	(9)	4	3	0	2	—
0.01 μ g	(9)	0	4	2	3	P < 0.2
0.03 μ g	(9)	0	3	1	4	P < 0.1
0.1 μ g	(9)	0	2	0	6	P < 0.025

* 1 : active wakefulness, 2 : standing/sitting with eyes open, 3 : standing motionless with eyes closed, and 4 : sitting motionless with head drooped (sleeping posture).

Results and Discussion

As shown in Table 1, GLP-1 (7-36) induced sleeping posture (posture of the score 4) at 30 min following i.c.v. injection in a dose-dependent fashion when compared with the saline control. It produced the entire prevention of posture of the score 1 (active behavior). The present result was in agreement with other report for feeding behavior of the neonatal chick by FURUSE *et al.* (1997c), who implied that GLP-1 may cause sedation or narcolepsy (sleep-like behavior).

In young adult hens, during the sleeping posture (head tucked under a wing) and the resting posture with eyes closed, there is nearly always electrophysiological sleep which points towards a good correlation (VAN LUIJTELAAR *et al.*, 1987). Thus, postures of the score 3 and 4 (GLP-1 induced sleep-like behavior) in the present result appear to be consider sleeping behavior on electrophysiology and GLP-1 is thought to have induce sleeping behavior in the neonatal chick. From the standpoint of the relationship between sleep-like behavior and feeding behavior, DENBOW *et al.* (1981) observed that i. c.v. injection of norepinephrine (NE) failed to stimulated food intake in the domestic fowl, presumably because it induced a narcoleptic response. Thus, there is the possibility that the anorexigenic effect of GLP-1 was due to GLP-1 induced sleep-like behavior by polyrria of NE. Indeed, central injection of fusaric acid (an inhibitor of dopamine- β -hydroxylase, a late-limiting enzyme in NE synthesis : RACZ *et al.*, 1985) which suppressed REM sleep in cats (SATO and TANAKA, 1973), attenuates the effect of GLP-1 on food intake (BUNGO *et al.*, unpublished data).

In conclusion, it is suggested that central administration of GLP-1 (7-36) amide may stimulate sleeping behavior in the neonatal chick.

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グルカゴン様ペプチド-1 の脳室内投与は新生ニワトリヒナの睡眠様行動を引き起こす

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グルカゴン様ペプチド-1 (GLP-1) の中枢投与によりラットとヒナの摂食量が減少することが報告されているが, ヒナにおける GLP-1 の摂食抑制機構は今だ明らかになっていない。本研究は, GLP-1 が新生ニワトリヒナの睡眠様行動を誘発するかどうかを明かにするために行った。脳室内に投与した 0.03 および 0.1 μg の GLP-1 (7-36) アミドは対照の生理的食塩水に比べてヒナの睡

眠様行動を引き起こすことが認められた。中枢神経系における GLP-1 は, ニワトリヒナの睡眠を誘発する因子の 1 つであることが示唆された。

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キーワード: グルカゴン様ペプチド-1 (GLP-1), 睡眠様行動, 脳室投与, 新生ニワトリヒナ