

新生ヒナにおけるグレリンの脳室投与は睡眠様行動を引き起こす

誌名	The journal of poultry science
ISSN	13467395
著者名	橘, 哲也 大串, 淳 古瀬, 充宏
発行元	Japan Poultry Science Association
巻/号	38巻4号
巻号補足	
掲載ページ	p. 358-363
発行年月	2001年10月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター

Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council
Secretariat



《Research Note》

Intracerebroventricular Injection of Ghrelin Induces Sleep-Like Behavior in Neonatal Chicks

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Ghrelin, a novel growth hormone (GH)-releasing acylated peptide, is recently isolated from rat stomach. This peptide stimulates the release of GH from the anterior pituitary through the GH secretagogue receptor and feeding in the rat. However, intracerebroventricular (ICV) injection of rat ghrelin inhibited feeding of chicks under both *ad libitum* feeding and fasting conditions (Furuse *et al.*, 2001). Thus, we have investigated the behavioral changes by ghrelin with special reference to sleep-like behavior of the neonatal chick in two experiments. Four behavioral categories were distinguished and scored as follows : 1, standing with eyes open ; 2, sitting with eyes open ; 3, standing with eyes closed ; and 4, sitting motionless with head drooped (sleeping posture). Firstly, ICV injection of 0, 1.25, 2.5 and 5 μ g of ghrelin was done to *ad libitum* fed birds. Secondly, several doses (0, 0.5, 1 and 2 μ g) of ghrelin were injected ICV into chicks previously deprived of food for 3 h. In both experiments, scores of ghrelin treated birds were higher than those of control birds. These results suggest that one of the central roles of ghrelin in the neonatal chick is induction of sleep.

Key words : ghrelin, intracerebroventricular, sleep-like behavior, chick

Introduction

Domestic chickens are precocial and start spontaneous feeding at hatch. In the neonatal stage, several factors that stimulate feeding in mammals have been investigated in the chick. These factors are neuropeptide Y (NPY), orexin-A, orexin-B, motilin, melanin-concentrating hormone and galanin, but except for NPY (Furuse *et al.*, 1997 a ; Bungo *et al.*, 2000 ; Kawakami *et al.*, 2000) these factors failed to stimulate feeding in the neonatal chick (Furuse *et al.*, 1999 ; Ando *et al.*, 2000). Thus, the mechanism for stimulatory effects on food intake in the neonatal chick may be specific. Especially the effect of ghrelin was interesting. Ghrelin is the newly purified peptide from the stomach (Kojima *et al.*, 1999) and stimulates feeding in the rat (Tschop *et al.*, 2000 ; Wren *et al.*, 2000). However, the central effect of ghrelin in chicks was reverse to

Received : April 20, 2001 Accepted : June 18, 2001

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those for rats. Intracerebroventricular(ly) (ICV) injection of rat ghrelin strongly suppressed food intake in chicks under both *ad libitum* and fasting conditions (Furuse *et al.*, 2001).

In the suppressive effect of peptides on feeding, two behavioral patterns have been observed in the neonatal chick. One is the motionless and narcoleptic behaviors induced by glucagon-like peptide-1 (Furuse *et al.*, 1997b ; Bungo *et al.*, 1999b). Another is active and exciting behaviors by corticotropin releasing factor (Furuse *et al.*, 1997b ; Ohgushi *et al.*, 2001).

To confirm the central effect of ghrelin, sleep-like behavior was determined under *ad libitum* feeding condition in the neonatal chick. Furthermore, we determined the sleep-like behavior after food deprivation to examine whether ghreline induces sleep-like behavior beyond the appetite strengthened by fasting.

Materials and Methods

Day-old male broiler chicks were purchased from a local hatchery (Mori Hatchery, Fukuoka, Japan). The birds were maintained in a room with 24-h lighting and at a temperature of 28°C. They were given free access to a commercial starter diet (Toyohashi Feeds and Mill Co. Ltd., Aichi, Japan). Before each experiment, body weight was measured and the chicks were distributed into experimental groups such that the average body weight within groups was as uniform as possible within the same experiment. The birds were reared individually. Birds of 2 days-old fed either *ad libitum* (experiment 1) or deprived of food for 3 h (experiment 2) were given the diet for 2 h immediately after the ghrelin injection.

Ghrelin (rat) was purchased from Peptide Institute, Inc. (Osaka, Japan). The peptide was dissolved in a 0.1% Evans Blue solution, which was prepared in 0.85% saline. Saline containing Evans Blue was used as a control (0 μ g level of ghrelin). The birds were injected ICV using a microsyringe according to the method of Davis *et al.* (1979) and Furuse *et al.* (1999) at a volume of 10 μ l. Injected doses of ghrelin were as follows : experiment 1, 0 (control), 1.25, 2.5, 5 μ g ; experiment 2, 0 (control), 0.5, 1, 2 μ g.

Behavioral patterns were observed at 120 min (experiment 1) or 30, 60 and 120 min (experiment 2) after the injection and classified into four categories. During these periods, chicks were freely given the diet and water. Posture scores were noted during feeding using a modification of the method of van Luijckelaar *et al.* (1987). Four behavioral categories were distinguished and scored as follows : 1, standing with eyes open ; 2, sitting with eyes open ; 3, standing with eyes closed ; and 4, sitting motionless with head drooped (sleeping posture).

At the end of the experiments, the birds were sacrificed with an overdose of pentobarbital, and the location of the injection site was verified. Data from individuals lacking dye in the lateral ventricle were deleted. The number of birds used for experiment 1 was as follows : control (0 μ g), 10 ; 1.25 μ g, 9 ; 2.5 μ g, 10 ; and 5 μ g, 9. The number of birds used for experiment 2 was as follows : control (0 μ g), 8 ; 0.5 μ g, 10 ; 1 μ g, 7 ; and 2 μ g, 8.

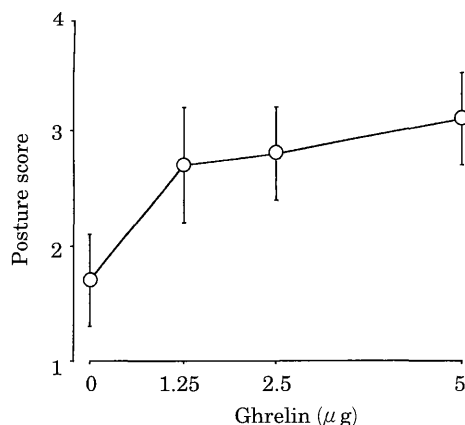


Fig. 1. Posture scores at 2 h following ICV injection of ghrelin in neonatal chicks under an ad libitum feeding condition. Values are means $\pm$ S.E.M. The number of birds used was : control (0 μ g), 10 ; 1.25 μ g, 9 ; 2.5 μ g, 10 ; and 5 μ g, 9. Four behavioral categories were distinguished and scored as : 1, standing with eyes open ; 2, sitting with eyes open ; 3, standing with eyes closed ; and 4, sitting motionless with head drooped (sleeping posture). Posture score = 2.01 (SE 0.32) + 0.26 (SE 0.12) X, $R^2 = 0.120$, $P < 0.05$.

Table 1. Effect of ghrelin on the various behavioral categories of the neonatal chick after 120 min following administration

Ghrelin	Number of chicks with the postures of score [#]			
	1	2	3	4
0 μ g	7	1	0	2
1.25 μ g	3	1	1	4
2.5 μ g	3	1	1	5
5 μ g	1	2	1	5

The number of chicks in each group was as follows : 0 μ g, 10 ; 1.25 μ g, 9 ; 2.5 μ g, 10 ; 5 μ g, 9.

[#] : Standing with eyes open, 2 : Sitting with eyes open, 3 : Standing with eyes closed, 4 : sitting motionless with head drooped (sleeping posture).

The results are presented as the mean $\pm$ S.E.M. Regression equations were fitted to the data.

Results and Discussion

Figure 1 shows the effect of ICV injection of 1.25, 2.5 and 5 μ g (0.38, 0.75 and 1.5 nmol) of ghrelin on posture scores of neonatal chicks fed *ad libitum* at 2 h after the injection. Table 1 summarizes the distribution of chicks in each group categorized by the posture scores. Central ghrelin induced sleep-like behavior in a dose-dependent

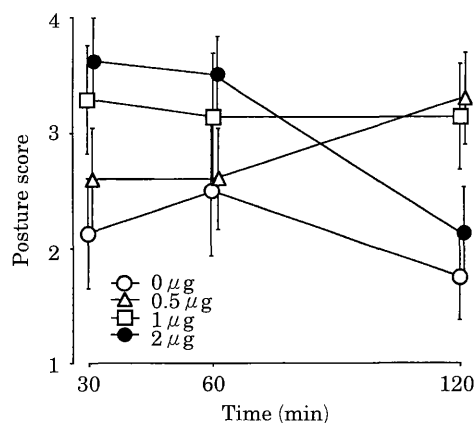


Fig. 2. Posture scores over 2h following ICV injection of ghrelin in neonatal chicks after 3 h fasting. Values are means $\pm$ S.E.M. The number of birds used was: control (0 μ g), 8; 0.5 μ g, 10; 1 μ g, 7; and 2 μ g, 8. Posture score at 30 min = 2.24 (SE 0.34) + 0.75 (SE 0.30) X, $R^2=0.170$, $P<0.05$. Posture score at 120 min = 1.88 (SE 0.39) + 3.02 (SE 1.03) X - 1.46 (SE 0.47) X², $R^2=0.242$, $P<0.01$.

Table 2. Influence of ghrelin on the various behavioral categories of the neonatal chick after 30, 60 and 120 min following administration

Time	Ghrelin	Number of chicks with the postures of score [#]			
		1	2	3	4
30 min	0 μ g	4	1	1	2
	0.5 μ g	4	0	2	4
	1 μ g	1	1	0	5
	2 μ g	1	0	0	7
60 min	0 μ g	4	0	0	4
	0.5 μ g	3	2	1	4
	1 μ g	2	0	0	5
	2 μ g	0	2	0	6
120 min	0 μ g	4	3	0	1
	0.5 μ g	2	0	1	7
	1 μ g	1	1	1	4
	2 μ g	3	2	2	1

The number of chicks in each group was as follows: 0 μ g, 8; 0.5 μ g, 10; 1 μ g, 7; 2 μ g, 8.

[#]: Standing with eyes open, 2: Sitting with eyes open, 3: Standing with eyes closed, 4: sitting motionless with head drooped (sleeping posture).

manner.

Figure 2 gives the effects of ghrelin on posture scores of 3h fasted chicks. In addition, Table 2 shows the distribution of chicks in each group categorized by the

posture scores. The doses were lowered from those used in experiment 1 since ghrelin strongly inhibited food intake under *ad libitum* feeding conditions (Furuse *et al.*, 2001). All of the groups ICV injected with ghrelin showed higher posture score than the control. Posture scores at 30 min showed a tendency to increase in a dose dependent fashion. At 2 h the posture score was curvilinearly modified by the central ghrelin.

These observations demonstrated that central administration of ghrelin induces sleep-like behavior in neonatal chicks. It is well known that ghrelin stimulates the release of growth hormone (GH) from the anterior pituitary through the GH secretagogue receptor (Kojima *et al.*, 1999). Earlier studies demonstrated that systemic administration of GH induced rapid eye movement (REM) sleep in several mammalian species (Drucker-Colin *et al.*, 1975 ; Mendelson *et al.*, 1980). It is possible that GH release stimulated by ghrelin might induce sleep-like behavior in neonatal chicks.

Ghrelin induced the sleep-like behavior during vigorous feeding after food deprivation, suggesting that central ghrelin may reduce food consumption by inducing sleep. This observation was similar to that induced by glucagon-like peptide-1 (Furuse *et al.*, 1997 b ; Bungo *et al.*, 1999 b), clonidine, an α_2 -receptor agonist (Bungo *et al.*, 1999 a) and morphine (Bungo *et al.*, 1999 c), but not by corticotropin releasing factor (Furuse *et al.*, 1997 b ; Ohgushi *et al.*, 2001). However, chicks were excited by central ghrelin after a short period (less than 30 min) and vocalized loudly. The reason for this was unclear. Further studies involving more behavioral tests and the anorexigenic mechanisms are necessary to elucidate the action of these peptides.

Acknowledgements

This study was supported by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan and Uehara Memorial Foundation.

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