Central effect of mammalian oxyntomodulin on food intake in non-fasted and fasted chicks

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Summary

Oxyntomodulin (OXM), a proglucagon-derived peptide, is a well known anorexigenic peptide found in the gut and brain of mammals. The present study was carried out to investigate the central effect of OXM on food intake in non-fasted and fasted Ross broiler chicks. At four weeks of age, a guide cannula was stereotaxically implanted into the right lateral ventricle of each bird. Two experiments were conducted on free-feeding (non-fasted) and 6-h fasted broilers. In each experiment, eight birds were used in a replicated 4 × 4 Latin square design. The birds were given an intracerebroventricular (ICV) injection of different doses of mammalian OXM. Intracerebroventricular injection of OXM at doses of 1.5, 3 and 6 nmol significantly ($P<0.05$) decreased food intake for 3 h post injection in both non-fasted and fasted chicks. It may be concluded that central OXM may exert a suppressive effect on food intake in chicks.

Key words: Oxyntomodulin, Food intake, Intracerebroventricular injection, Chick

Introduction

Neurochemical control of food intake has been investigated in birds for the past 30 years, and a plenty of neurotransmitters discovered to suppress or to stimulate food intake when given into the brain in chicks (Denbow, 1994).

Oxyntomodulin (OXM) and glucagon-like peptide-1 (GLP-1) are proglucagon-derived gut hormones which are found in the brain, and released in response to nutritional stimuli (Chaudhri *et al*., 2008). The avian proglucagon system has recently been described (Richards and McMurtry, 2009). According to this report, the avian OXM is a 55 - amino acid peptide segment derived from proglucagon precursor that is composed of glucagon and intervening peptide-1 (IP-1). Because the chicken precursors contain an elongated IP-1, oxyntomodulin would be larger in the chicken compared to the 37 amino acids peptide produced by mammals (Richards and McMurtry, 2009). However, the 37 amino acids peptide of OXM is widely conserved among different mammals and birds as revealed by the multiple sequence alignment program ClustalW (Larkin *et al*., 2007). It has been shown that OXM and GLP-1 have reducing effects on food intake and body weight. Central and systemic injections of OXM and GLP-1 to rodents decreased food intake (Baggio *et al*., 2004; Dakin *et al*., 2004), and similarly, both OXM (Cohen *et al*., 2003) and GLP-1 (Gutzwiller *et al*., 1999) reduced appetite and energy intake in humans.

The presence of proglucagon mRNA in the brain stem of chicks has been reported (Tachibana *et al*., 2005). The anorexigenic action of glucagons (Honda *et al*., 2007) and GLP-1 (Furuse *et al*., 1997a) has been studied in neonatal chicks, however, there is

only one study regarding the anorexigenic effect of OXM in neonatal chicks (Cline *et al*., 2008). The physiological state of the animal influences the food intake response to central neurotransmitters. For example, it has been reported that intracerebroventricular (ICV) injection of serotonin decreases food intake in fed broilers, but is without effect in fasted broilers (Denbow *et al*., 1982). In order to further study the biological actions of OXM in birds, the effect of ICV injection of OXM on food intake was investigated in both fasted and non-fasted broiler chicks in the current investigation.

Materials and Methods

Day-old male Ross broiler chicks (Mahan Co., Kerman, Iran) were reared in standard conditions with continuous light. The birds were provided a mash diet (20% protein, 2864 kcal/kg metabolizable energy) and water *ad libitum*. At approximately 3 weeks of age, the birds were transferred into individual cages, each with an individual feed and water supply, and room temperature was maintained between 21 and 23°C. All experiments were conducted after institutional approval of animal use committee of Shahid Bahonar University of Kerman.

At 4 weeks of age, the chicks were anesthetized with sodium pentobarbital (15 mg/kg, IV) and diazepam (3 mg/kg, IM), and then a 23-gauge thin-walled stainless steel guide cannula was stereotaxically implanted into the right lateral cerebral ventricle. The cannula was positioned 6.7 mm anterior to the bregma, 0.7 mm lateral to the midline and 3.5-4 mm below the surface of the durra matter (Denbow *et al*., 1982) with head oriented as described previously (Van Tienhoven and Juhasz, 1962). The location of the cannula into the lateral ventricle was verified by ICV injection of 150 ng of angiotensin Π (Tocris bioscience, UK). Only those birds that robustly drank water after the injection were used for the study. In order to minimize the effects of handling stress, the birds were handled daily during the 1-week recovery period.

All solutions were injected at 1-day intervals so that each bird received each solution during the 4-day test period (Table 1).

Mammalian OXM (Tocris bioscience, UK) solutions were prepared in a pyrogen-free 0.9% NaCl solution which served as the control. Injections were made with a 27-gauge, thin-walled stainless steel injection cannula which extended 1.0 mm beyond the guide cannula. The injection cannula was connected to a 10 µl Hamilton syringe via PE tubing which had been kept in 70% ethanol. Before the injections, the birds were removed from their individual cages and restrained by hand, then they were put back into their cages after injections. Solutions were injected over a 30-s period, and the injection cannula remained in place for an additional 30 s before removal.

Two experiments were conducted in this study. In each experiment, eight birds were used in a replicated 4 × 4 Latin square design in which the birds and days were blocking factors (Table 1).

In experiment 1, ICV injection of OXM at doses of 0, 1.5, 3 and 6 nmol was performed in non-fasted birds. Solutions were injected in a volume of 10 µl. Experiment 2 was the same as experiment 1, except that the animals were fasted for 6 h. All injections took place between 10:00 and 11:00 h. After treatment, chicks were returned to their individual cages, pre-weighed feeders were placed, and cumulative feed intake was measured at 30, 60, 90, 120, 150 and 180 min.

Cumulative feed intake was presented as mean ± SEM. The statistical design used was a repeated Latin square with two

Table 1: Procedure of receiving solutions by chicks in each experiment

Days	Chicks 1 and 2	Chicks 3 and 4	Chicks 5 and 6	Chicks 7 and 8
1	NaCl	1.5 nmol OXM	3 nmol OXM	6 nmol OXM
2	1.5 nmol OXM	NaCl	6 nmol OXM	3 nmol OXM
3	3 nmol OXM	6 nmol OXM	NaCl	1.5 nmol OXM
4	6 nmol OXM	3 nmol OXM	1.5 nmol OXM	NaCl

OXM: Oxyntomodulin

squares, four treatments, and six measurements overtime having in total 192 experimental units. Data were analyzed using SAS software and the means of interactive effect between the treatment and the time period was calculated. For treatment showing a significant main effect by ANOVA, means have been compared by a post-hoc LSD test. Statistical significance was set at $p<0.05$ for all experiments.

Results

The results of this study indicated that an ICV injection of OXM at doses of 1.5, 3.0 and 6.0 nmol causes a reduction in feed consumption in free-feeding (non-fasted) broilers from 30 to 180 min after injection (Fig. 1). ICV injection of all 3 doses of OXM also caused a significant decrease in feed intake of fasted broilers in all time periods (Fig. 2). In both experiments, the pattern of the OXM dose effect seems to be "all-or-none". This means that all OXM doses were equally effective, versus control. Generally, it appears that the effect of OXM is fairly rapid, showing the greatest effect 30 min after ICV injection in both physiological states (although study 1 did not show significant ANOVA results until the 90-minute time point). Also, in both experiments, the birds treated with OXM did not eat more after 30 min post injection. Separation of food consumption from controls to OXM treatments continued to increase as time progressed (Figs. 1 and 2). This could mean that central OXM exerts a sustaining suppression of food intake.

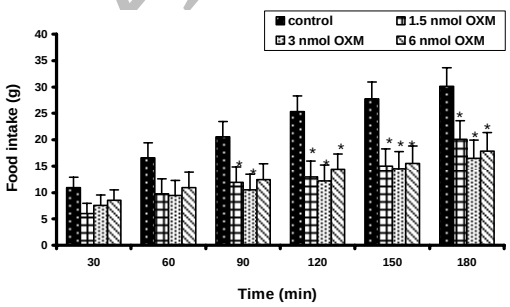

Fig. 1: Effect of intracerebroventricular injection of different doses of oxyntomodulin on cumulative food intake in non-fasted broiler cockerels. *Significant difference with control group, (P<0.05)

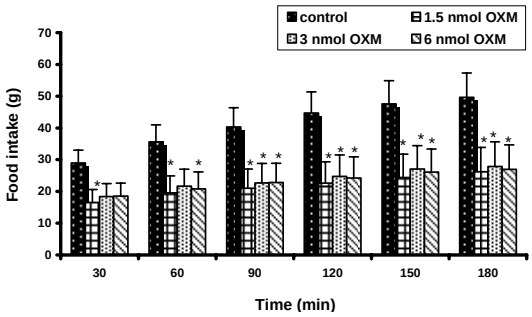

Fig. 2: Effect of intracerebroventricular injection of different doses of oxyntomodulin on cumulative food intake in fasted broiler cockerels. *Significant difference with control group, (P<0.05)

Discussion

The doses of OXM used for neonatal chicks (Cline *et al*., 2008) were lower than those which were active in the current study. The reason for this is not clear but there is one possible explanation; age may be important, because one-month chicks were used in the present study and 2-day chicks were used in the neonatal chick study. Sensitivity to OXM in the brain may be altered by aging, as young animals are more sensitive. The mammalian OXM was used since the sequence of avian OXM was not determined at the time of this investigation. However, the OXM peptide used in the current study is highly conserved among different mammals and chicks (Larkin *et al*., 2007), and the 55 amino acid avian OXM has 18 additional residues at the C-terminal (Richards and McMurtry, 2009). The 18 amino acid extra segment on avian OXM is a part of the intervening peptide 1 (IP-1) that divides glucagon from GLP-1 in the proglucagon protein precursor, and probably does not interfere with OXM binding to receptor.

The anorexigenic effect of OXM in our study is consistent with a recently published study (Cline *et al*., 2008) referring to neonatal chicks, and with the results of similar experiments to do with rodents (Dakin *et al*., 2001) and human (Cohen *et al*., 2003). The current result is also similar to the anorexigenic response induced by ICV injection of glucagons (Honda *et al*., 2007) and GLP-1 (Furuse *et al*., 1997a) in neonatal chicks and ICV injection of GLP-1

in rodents (Turton *et al*., 1996).

The proglucagon mRNA expression has been observed in the brainstem and telencephalon of chicks (Tachibana *et al*., 2005). This result demonstrates that proglucagon-derived peptides including OXM may be present in the chick brain. Investigations suggest that OXM mediates its actions via the GLP-1 receptor in rat (Fehmann *et al*., 1994). In the brain of birds, OXM may possibly signal through the GLP-1 receptor (GLP-1R) or glucagon receptor (GLP) since glucagon is a component of the OXM (Richards and McMurtry, 2009). The sequences of GLP-1R and GLP have significant similarity between different mammals (human, mouse and rat) and chicks. (ClustalW output results not shown). Thus, the mammalian OXM can be actively bound to the avian GLP1-R or GLP.

In conclusion, according to our data it seems that a 37 amino acid mammalian OXM acts on the brain of fasted and non-fasted broilers to inhibit food intake.

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