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Research article

Central injection of a synthetic chicken partial leptin peptide does not affect food intake in chicks

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HIGHLIGHTS

- The role of chicken leptin in appetite regulation is unknown.
- Central injection of chicken leptin did not affect food intake in chicks.
- Chicken leptin did not affect water intake.
- Most behaviors were not affected by leptin injection.
- Results suggest that chicken leptin does not regulate food intake in chicks.

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ABSTRACT

Leptin is an adipose tissue-derived hormone in mammals that plays an important role in whole body energy balance via its inhibitory effects on food intake mediated through the hypothalamus. Chicken leptin has a low sequence homology to mammalian leptin and its role in appetite regulation is not reported; hence the objective of this study was to determine effects of central injection of chicken leptin on food and water intake and associated behaviors in chicks. Chicks were intracerebroventricularly injected with 0 (vehicle), 0.3, 1.0, or 3.0 nmol of a synthetic chicken leptin partial peptide and food and water intake were monitored. There were no effects observed and a second experiment was conducted to evaluate food and water intake at higher doses; after injection of 0, 2.5, 5.0, or 10.0 nmol leptin. Again, there were no effects on food or water intake. In the third experiment, behaviors were analyzed during the first 30 min post-injection of vehicle or 10 nmol leptin. At 5 min post-injection, vehicle-injected chicks spent more time sitting than leptin-injected chicks. A wide dose range was evaluated however, the absence of an effect on food intake or behavior suggests that the chicken leptin peptide that was tested does not mediate effects on appetite in the brain and that chicken leptin likely has a different physiological role in birds than in mammals.

1. Introduction

Leptin is a protein intensively studied for its role in appetite and whole body energy balance in mammals. A null mutation in the leptin gene leads to extreme obesity in *ob/ob* mice [1], and mutation of the leptin receptor (LEPR) results in diabetes in *db/db* mice [2]. Soon after the first report of leptin in 1994, it was demonstrated that this hormone plays an important role in regulating body weight [3] via the hypothalamus to inhibit feeding behavior [4,5].

In 1998, a group reported the cloning of the chicken leptin gene, with 97%, 96% and 83% similarity to the mouse, rat and human amino acid sequences, respectively [6]. The following year, another group reported that they were unable to amplify this putative chicken leptin sequence by PCR [7]. The existence of chicken leptin was questioned although chicken LEPR was identified [8,9]. Central injection of human leptin into chickens was associated with a reduction in food intake, suggesting that receptor function is conserved in avians [10]. However, central injection of mouse leptin into broiler and Leghorn chicks did not have any effects on food intake [11].

Partial sequences from Japanese quail (*Coturnix japonica*) [12] and RNA-seq data from the Short Read Archives led to the discovery of the chicken leptin sequence [13]. Authors noted that identification had likely been hindered by the high guanine-cytosine content (~70%) in a genomic region with repetitive and palindromic sequences, coupled to low sequence identity (~30% amino acids) with mammalian leptin and low expression in adipose tissue, the region of highest expression in mammals [13]. These findings raise

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the question of whether avian leptin plays a similar role in regulating energy balance through appetite regulation as in mammals. Thus, the objective of this study was to evaluate the effect of chicken leptin on food and water intake and associated behaviors in chicks.

2. Materials and methods

2.1. Animals

Hubbard x Cobb-500 chicks (*Gallus gallus*) were obtained from a commercial hatchery on the morning of hatch. Chicks were caged individually at 30 ± 1 °C and 50 ± 5% relative humidity and provided fresh water and a mash diet (22% crude protein 3000 kcal ME/kg), the composition of which has been reported [14]. Experiments were conducted between 08:00 and 11:00 and each experiment used chicks from separate hatches. All procedures were carried out in strict accordance with the National Research Council publication, Guide for Care and Use of Laboratory Animals and were approved by the Virginia Polytechnic Institute and State University Institutional Animal Care and Use committee.

2.2. Intracerebroventricular (ICV) injection of chicken leptin

On day 4 post-hatch, chicks were fasted for 180 min and then intracerebroventricularly injected, using an adapted method [15]. The head of the chick was briefly inserted into a restraining device that left the cranium exposed and allowed for free-hand injection. Injection coordinates were 3 mm anterior to the coronal suture, 1 mm lateral from the sagittal suture, and 2 mm deep targeting the left lateral ventricle. Anatomical landmarks were determined visually and by palpation. Injection depth was controlled by placing a plastic tubing sheath over the needle. The needle remained *in vivo* in the un-anaesthetized chick for 5 s to reduce backflow

Based on the published chicken leptin sequence [13], the following peptide was synthesized (no N- or C-terminal modifications) by AnaSpec (San Jose, CA, USA): PRAEKLRADARSLSRTLSARLGDVKPPPPSLR. These amino acids correspond to region 22–56 of human leptin that when synthesized and injected into the right lateral ventricle, inhibited feeding in rats [16]. It should be noted that the *Gallus gallus* sequence reported lacks the N-terminal leader sequence and there is a gap at the final two amino acids that correspond to human 22–56 [13]. The peptide was dissolved in avian artificial cerebrospinal fluid to act as a vehicle, with an injection volume of 5 μL, with 0.06% Evan's Blue dye to determine injection site location. After data collection, each bird was decapitated and the brain sectioned to determine site of injection. Any chick without dye present was eliminated from analysis. The sex of each chick was determined visually by dissection.

2.3. Experiment 1: food and water intake in low dose-injected chicks

After a 180 min fast, chicks received an ICV injection of 0 (vehicle), 0.3, 1.0, or 3.0 nmol of chicken leptin. After injection, chicks were returned to their cages and provided ad libitum access to food and water. Food and water were measured every 30 min for 180 min post-injection. Data were analyzed by analysis of variance (ANOVA) within each time point. The statistical model included the effects of leptin dose, sex, and the interaction between them. Sex and the interaction were non-significant, thus sex was removed from the model. Significant main effects of treatment were separated using Tukey's test. The number of chicks for each experiment are shown in the figure captions and for all experiments significance was set at $P < 0.05$.

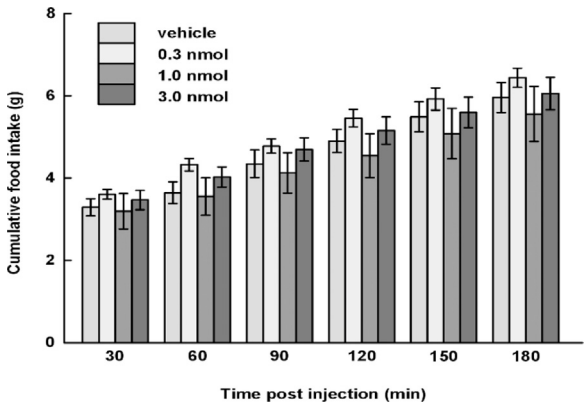

Fig. 1. Cumulative food intake. Data are shown as means ± standard errors (n = 10 per treatment). At 4 days post-hatch, chicks were injected intracerebroventricularly with 0 (vehicle), 0.3, 1.0, or 3.0 nmol chicken leptin and food intake was measured for 180 min post-injection.

2.4. Experiment 2: food and water intake in high dose-injected chicks

Methods were the same as for 2.3 except that chicks were assigned to receive 0, 2.5, 5, or 10 nmol chicken leptin.

2.5. Experiment 3: behavior analysis

Chicks were fasted for 180 min and randomly assigned to receive ICV injection of either vehicle or 10 nmol chicken leptin. Immediately following injection, chicks were placed in a 290 × 290 mm acrylic recording arena with food and water containers in diagonal corners. The chicks were simultaneously and automatically recorded from three angles for 30 min post-injection on DVD and data were analyzed in 5 min intervals using ANY-maze behavioral analysis software (Stoelting, Wood Dale, IL). The following traits were measured: amount of time spent standing, sitting, preening, or in deep rest in seconds (time-type behaviors), and the number of jumps, steps, food and exploratory pecks, drinks, defecations, and chirps (count-type behaviors). Deep rest was defined as the eyes being closed for more than 3 s starting 3 s after eye closure. Preening was defined as trimming or dressing of down with the beak. Food pecks were defined as pecks in the food container while other pecks were classified as exploratory pecks. Drinks were defined as the chick dipping its beak in water, then raising and extending its head to swallow. Data were analyzed by the Mann-Whitney *U* test due to variance.

3. Results

3.1. Food and water intake

There were no effects on food (Fig. 1) or water (Fig. 2) intake in chicks after injection of 0.3, 1.0, or 3.0 nmol chicken leptin. Food (Fig. 3) and water (Fig. 4) intake were not affected by 2.5, 5.0, or 10.0 nmol chicken leptin.

3.2. Behavioral analyses

There were no effects of leptin injection of count-type behaviors, including the numbers of feeding pecks, jumps, exploratory pecks, escape attempts, drinking pecks, defecations, or steps (Table 1). At 5 min post-injection, vehicle-injected chicks spent more time sitting than leptin-injected chicks (Table 2). Other timed behaviors were not affected by treatment at any of the time points.