Behavioural Brain Research 208 (2010) 650–654

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Short communication

The threshold of amylin-induced anorexia is lower in chicks selected for low compared to high juvenile body weight

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ARTICLE INFO

Article history:
Received 1 December 2009
Received in revised form
17 December 2009
Accepted 21 December 2009
Available online 29 December 2009

Keywords:
Amylin
Chick
Hypothalamus
Food intake
Obesity
Anorexia
Behavior
c-Fos

ABSTRACT

Chicks that have undergone long-term selection for low body weight responded to intracerebroventricular amylin injection with reduced food intake at a dose considerably lower and with a greater magnitude suppression than those selected for high body weight. Behaviors unrelated to ingestion were not affected. These data support the thesis of correlated amylin system responses to selection for low or high body weight, with possible implications to other species.

Amylin is receiving increasing attention in appetite biology. Co-secreted with insulin from pancreatic beta cells [15] in response to ingestion of a meal [2], it is well documented that amylin decreases food intake across a range of species [7,21,10]. Accordingly, because its basal concentration is higher in obese humans, it is primarily associated with the obese phenotype [16]. When centrally administered, amylin is more potent than other members of its family [22] such as calcitonin and calcitonin gene-related peptide, which also elicit anorexigenic responses [10,23].

Amylin decreases food intake in chicks when administered centrally or peripherally, and although it decreases c-Fos reactivity in the hunger-associated lateral hypothalamus, it does not affect c-Fos expression in the ventromedial hypothalamus, which is classically associated with satiety [7]. Furthermore, we demonstrated that central amylin increases transit time through the alimentary canal, anxious-like behavior, and plasma corticosterone concentration [7].

Rodent studies have also demonstrated roles for amylin in decreasing meal size [21] and frequency [24]. Diet-induced obese rats respond to amylin with decreased food intake and weight gain while increasing energy expenditure [25]. However, to our knowledge these effects have not been reported in a polygenic model of obesity, nor have the anorectic effects of amylin been studied simultaneously in animal models of anorexia and obesity. The study reported here aimed to determine if amylin differentially affects food intake and behavior in lines of chickens that have undergone long-term selection for high and low body weight and that are hypo- and hyperphagic.

The chicks used in this study are the result of long-term divergent selection for low (LWS) or high (HWS) body weight at 56 days-of-age from a White Plymouth Rock founder population that consisted of crosses of 7 inbred lines and were maintained as closed populations [13,26,19]. Eggs obtained from age contemporary parents from S_{48} and S_{49} generation parental stocks were incubated in the same machine. After hatch, chicks were group-caged for 2 days, then individually in a room at $30 \pm 2\,^{\circ}C$ and $50 \pm 5\%$ relative humidity where they had *ad libitum* access to a mash diet (20% crude protein, 2685 kcal ME/kg) and tap water. The individual cages allowed visual and auditory contact with other chicks

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doi:10.1016/j.bbr.2009.12.032

(unless otherwise indicated). Chicks were handled twice daily to adapt to handling. All trials were conducted between 11:00 and 16:00 h using 5-day post-hatch chicks. Data in each experiment were recorded from both lines concurrently and injections were performed sequentially, LWS, HWS, LWS, HWS and so forth. Experimental procedures were performed according to the National Research Council publication, Guide for Care and Use of Laboratory Animals and were approved by the Radford University Institutional Animal Care and Use Committee.

Chicks were injected using a method adapted from Davis et al. [9] that does not appear to induce physiological stress [14]. 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 at injection depth for 5 s post-injection to reduce backflow. Chicks were assigned to treatments at random. Amylin (American Peptide Co., Sunnyvale, CA, USA) was dissolved in artificial cerebrospinal fluid as a vehicle for a total injection volume of 5 μL with 0.1% Evans Blue dye to facilitate injection site localization. The sequence of rat amylin injected (KCNTATCATQRLANFLVRSSNNLGPVLPPT-NVGSNTY) has 82% sequence identity to that of chicken [21]. After data collection, the chicks were decapitated and their heads sectioned coronally to determine site of injection. Any chick without dye present in the lateral ventricle system was eliminated from analysis.

In Experiment 1, chicks from the S_{48} generation from each line were fasted for 180 min prior to injection to intensify the perception of hunger. They were randomly assigned to receive 0 (vehicle only), 128 (0.5 μg), 255 (1.0 μg) or 510 (2.0 μg) pmol amylin by ICV injection. After injection, chicks were returned to their individual cages and given *ad libitum* access to both food and water, with individual food and water containers weighed (0.01 g) every 30 min for 180 min post-injection. Data were analyzed using analysis of variance (ANOVA) at each time point. The model included line, amylin dose and the line by amylin dose interaction. When the interaction was significant ($P < 0.05$), data were analyzed within each line for the effect of amylin dose using Tukey's method of multiple comparisons. The LWS and HWS lines consume inherently different amounts of food due to differences in body size. Therefore, food and water intake data were normalized per body weight at each time point. This conversion was made by dividing the amount of feed each chick consumed by its body weight (0.01 g) immediately prior to its ICV injection and multiplying by 100.

In Experiment 2, chicks from the S_{49} generation from each line were kept in individual cages with auditory but not visual contact (to reduce isolation stress during behavior measurement) from 2 days post-hatch. They were randomly assigned to receive either vehicle or 255 pmol amylin, a dose that exceeded threshold in both lines in Experiment 1, by ICV injection. Following a 180 min fast they were injected and immediately placed in a 290 mm × 290 mm acrylic recording arena with food and water containers in diagonal corners. Chicks were simultaneously and automatically recorded from three angles for 30 min post-injection on DVD and data were analyzed in 10 min intervals using ANY-maze behavioral analysis software (Stoelting, Wood Dale, IL). Locomotion (m traveled), time spent (s) standing, sitting, preening, perching or in deep rest, and the number of jumps, food and exploratory pecks, drinks, defecations and escape attempts were recorded. Food pecks were defined as pecks within the food container, whereas all other pecks were considered as exploratory. Drinks were defined as the chick dipping its beak in water, then raising and extending its head to swallow. Preening was defined as trimming or dressing of down with the beak. Deep rest was defined as the eyes closed for greater than 3 s, starting 3 s after eye closure. The statistical model used was the same as that for Experiment 1.

In Experiment 1, amylin reduced food intake in both lines (Fig. 1), and the line by amylin dose interaction was significant. The interaction occurred because the threshold of amylin-induced anorexia was lower for line LWS than line HWS chicks. That is, in line LWS all doses of amylin tested reduced food intake nearly 50%. In line HWS however, 128 pmol did not reduce food intake whereas 255 and 510 pmol of amylin caused only a 25% reduction in food intake. There were 12–14 chicks in the line LWS and 17–18 in HWS per amylin dose.

These results demonstrate that line LWS has a lower threshold of amylin-induced anorexia than line HWS. The decreased food intake pattern in chicks from line HWS more closely resembles that of Cobb-500 chicks than that of line LWS [7] and the decreased food intake is consistent with that reported for rodents [21,10]. Therefore, selection for juvenile body weight may have favored a gain

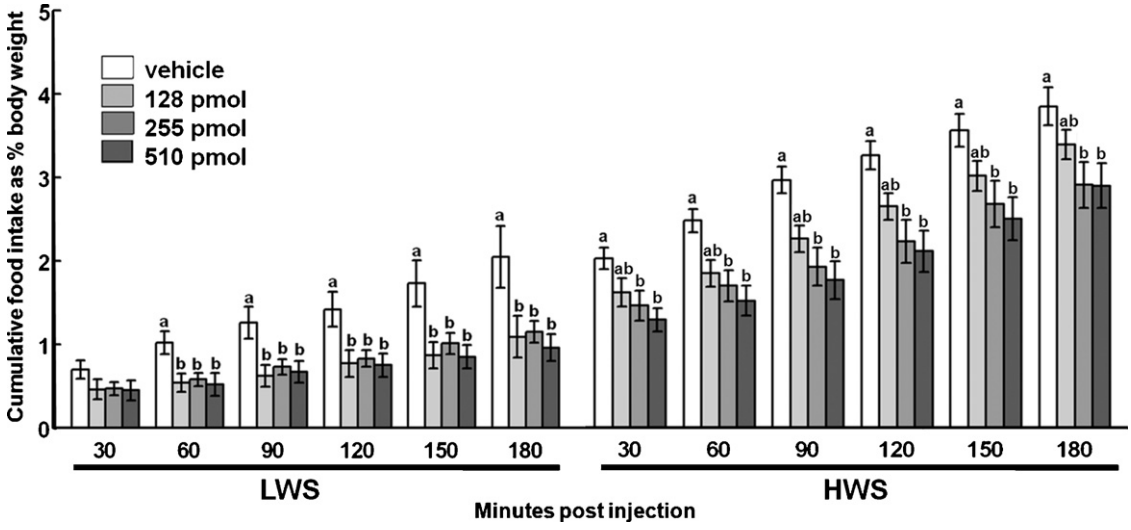

Fig. 1. Cumulative food intake expressed as percent body weight following intracerebroventricular injection of amylin in low (LWS) and high (HWS) body weight long-term divergently selected lines of chicks (Experiment 1). Values are means ± SE; bars with different letters are different from each other within a time point ($P < 0.05$). $n = 12–14$ chicks in the line LWS and 17–18 in HWS per amylin dose.