General and Comparative Endocrinology 224 (2015) 278–282

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Short Communication

Beta-cell-tropin is associated with short-term stimulation of food intake in chicks

Steven L. Shipp, Marissa L. Smith, Elizabeth R. Gilbert, Mark A. Cline *

Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA

ARTICLE INFO

Article history:
Received 20 March 2015
Revised 20 July 2015
Accepted 1 August 2015
Available online 4 August 2015

Keywords:
Beta-cell-tropin
Hypothalamus
Food intake
Chick

ABSTRACT

Beta-cell-tropin, a peptide derived from adrenocorticotropic hormone, is an insulin secretagogue. When centrally injected, it increases food intake in rats, but its appetite-associated effects have not been reported in any other species. Thus, the present study was designed to evaluate the effects of central beta-cell-tropin on appetite-associated parameters in an alternative vertebrate model, the chick. Central injection of 2 or 4 nmol beta-cell-tropin increased food intake for 60 min. Whole hypothalamus was collected at 60 min post-injection, and real-time PCR performed to measure mRNA abundance of agouti- related peptide, corticotropin releasing factor, galanin, melanin concentrating hormone, neuropeptide Y, orexin, prohormone convertase 2, pro-opiomelanocortin, peroxisome proliferator-activated receptor γ, urotensin 2, and visfatin, not one of which were affected by beta-cell-tropin treatment. Results demonstrate that beta-cell-tropin is associated with short-term stimulation of food intake.

1. Introduction

Beta-cell-tropin (βCT), a peptide first identified in the pituitary gland of lean and genetically obese mice (ob/ob) (Beloff-Chain et al., 1975), functions mainly as an insulin secretagogue (Beloff-Chain et al., 1980; Billingham et al., 1982) and lipogenic factor (Beloff-Chain et al., 1982; Watkinson and Beloff-Chain, 1984) in the periphery. Plasma concentrations are elevated in obese rhesus monkeys (Morton et al., 1992), mice, and rats (Morton et al., 1991). βCT is thought be involved in the development of hyperinsulinemia and obesity (Beloff-Chain et al., 1981). βCT also has other physiological functions, as it is elevated during early and mid-early pregnancy in rats (Davenport et al., 1995), is found in human plasma (Salvatoni et al., 1986), and has changes in plasma concentration in response to diet and fasting in human type II diabetic patients (Levy et al., 1992).

βCT is derived from adrenocorticotropic hormone (ACTH), and is also referred to as ACTH 22–39 (Beloff-Chain et al., 1983). While both ACTH and βCT are found in high concentrations in the pituitary, ACTH is also found in the amygdala, cerebral cortex, cerebellum, brain stem and arcuate nucleus of the hypothalamus (Civelli et al., 1982; Csiffary et al., 1990; Palkovits et al., 1987; Pilcher and Joseph, 1984), and ACTH can be processed into many different peptides in the brain, including βCT (Wang et al., 1983).

βCT is also involved in food intake; the only study of βCT's effect on food intake in any species demonstrated that central injection after a 16 h fast causes an increase in food intake in rats (Al-Barazanji et al., 2001). To evaluate the effects of βCT on food intake in chicks, we administered βCT through intracerebroventricular (ICV) injection, and measured food and water intake for 180 min post injection. To evaluate effects on the hypothalamus, because it is thought to be a major controller of food intake, mRNA abundance of appetite-associated factors was measured in the hypothalamus.

2. Materials and methods

2.1. Animals

Unsexed Hubbard × 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 with free access to a mash diet (21.5% crude protein and 3000 kcal ME/kg) and tap water. All experiments were conducted between 08:00 and 11:00 using 4 day post hatch chicks and each experiment used chicks from separate hatches. All experimental procedures were performed according to 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.

* Corresponding author.
E-mail address: macline2@vt.edu (M.A. Cline).

http://dx.doi.org/10.1016/j.ygcen.2015.08.004
0016-6480/

2.2. Intracerebroventricular (ICV) injection

Intracerebroventricular injections were performed using methods adapted from Davis et al. (1979). The head of the chick was momentarily 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-anesthetized chick for 5 s to reduce backflow. Human ACTH 22–39 (American Peptide Company, Sunnyvale, CA, Molecular weight: 1985.1) was dissolved in artificial cerebrospinal fluid with 0.06% Evan's Blue dye to confirm injection site and 5 μL injected. The sequence of human βCT injected (VYPNGAEDESAEAFPEF) has 88.9% sequence identity to chicken βCT (Hayashi et al., 1991). After data collection, each bird was decapitated and the brain sectioned to determine site of injection. Chicks without dye present were eliminated from the analysis. The sex of each chick was determined visually by dissection.

2.3. Experiment 1: food and water intake in chicks fed ad libitum

Chicks were randomly assigned to receive an ICV injection of 0 (vehicle), 1, 2, or 4 nmol βCT. Immediately following injection, chicks were returned to their cages with access to food and water *ad libitum*. Food and water were measured every 30 min for 180 min post injection. Water weight was converted to volume (mL; 1 g = 1 mL). Data were analyzed by analysis of variance (ANOVA) at each time point using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). The model included βCT dose, sex and the interaction of sex with βCT dose. Sex and the interaction of sex and βCT dose were not significant and eliminated from the model (and the effect of sex was not tested in subsequent experiments). Tukey's method of multiple comparisons was used to separate the means at each time period. For this and all other experiments, statistical significance was set at $P < 0.05$. The number of chicks used in each experiment is listed in figure legends.

2.4. Experiment 2: total RNA isolation and real time PCR

Each chick was randomly assigned to receive vehicle or 2 nmol βCT by ICV injection. Sixty minutes following injection, each chick was deeply anesthetized with sodium pentobarbital via cardiopuncture, decapitated, and brain removed. The inverted brain was submerged in liquid nitrogen such that the most ventral aspect of the optic lobe was level with the surface of the liquid nitrogen for 11 s. Perpendicular to the midline suture, a cut was made at the septopallio-mesencephalic tract and at the third cranial nerves. At 2.0 mm parallel to the midline, two cuts were made. The final cut was made from the anterior commissure to 1.0 mm ventral to the posterior commissure. This block comprising the hypothalamus was stored in RNAlater (Qiagen) and homogenized using 5 mm stainless steel beads (Qiagen) and 1 ml Isol Lysis reagent (5-Prime, USA) for 2 × 2 min at 20 Hz with a Tissue Lyser II (Qiagen). After incubation and centrifugation for 10 min for 12,000×*g* at 4 °C, the supernatant was removed and total RNA separated, following the manufacturer's instructions (5-Prime). Following the step of addition to 70% ethanol, mixtures were transferred to spin columns and total RNA purified using the RNeasy Mini Kit (Qiagen, USA). The eluted total RNA samples were evaluated for integrity by agarose-formaldehyde gel electrophoresis and concentration and purity assessed by spectrophotometry at 260/280/230 nm.

Single-strand cDNA was synthesized from 200 ng total RNA in 20 μL reactions with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA), following the manufacturer's instructions. Reactions were performed under the following conditions: 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 min. Primers for real time PCR were designed with Primer Express v3.0 (Applied Biosystems) and are listed in Table 1. Amplification efficiency was validated for all primer pairs before use (95–100% efficiency). Real time PCR reactions were performed in duplicate with Fast SYBR Green Master Mix (Applied Biosystems, USA) and 10-fold diluted cDNA using a 7500 Fast instrument (Applied Biosystems). PCR was performed under the following conditions: 95 °C for 20 s and 40 cycles of 90 °C for 3 s plus 60 °C for 30 s. A dissociation step consisting of 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s and 60 °C for 15 s was performed at the end of each PCR reaction to ensure amplicon specificity.

Data were analyzed using the ΔΔCT method, where ΔCT = CT target gene − CT actin, and ΔΔCT = ΔCT target sample − ΔCT calibrator (Schmittgen and Livak, 2008). Actin served as the endogenous control and the average of the vehicle-treated chicks served as the calibrator sample. Relative quantities, calculated as $2^{-\Delta\Delta CT}$, were used for ANOVA with the model including the main effect of treatment.

3. Results

3.1. Experiment 1: food and water intake in chicks fed ad libitum

Chicks injected with 2 and 4 nmol βCT increased their food intake (Fig. 1) starting at 30 min and lasting 60 min. Food intake was not affected in chicks injected with 1 nmol βCT, and was not affected by any dose post 60 min. Water intake was not affected by βCT at any time point or dose (Fig. 2).

3.2. Experiment 2: hypothalamic mRNA abundance of appetite-associated factors

Abundance of agouti-related peptide (AgRP), corticotropin releasing factor (CRF), galanin (GAL), melanin concentrating hormone (MCH), neuropeptide Y (NPY), orexin (ORX), prohormone convertase 2 (PC2), pro-opiomelanocortin (POMC), peroxisome proliferator-activated receptor γ (PPARγ), urotensin 2 (UT), and visfatin (VIS) mRNA was not affected at 1 h post βCT injection (see Fig. 3).

4. Discussion

In the present study, βCT was associated with short-term increased food intake in chicks. Under *ad libitum* conditions rats do not increase their food intake in response to βCT, however, 2.5 nmol βCT following a 16 h fast significantly increases food intake after 6 and 8 h post injection (Al-Barazanji et al., 2001). Chick food intake was increased at 30 min under *ad libitum* conditions. While food intake was measured at 1, 2, 3, 4, 5, 6, 8, and 24 h in the rat study, food intake was measured every 30 min for 180 min in the present study. Although the effect on food intake did not persist for the same duration as in rats, the effect on food intake in chicks occurred faster. The lowest efficacious dose for chicks was 2 nmol, thus the threshold dose is somewhere between 1 and 2 nmol. The rat study used a single dose of 2.5 nmol, thus a comparison of dose threshold responses between species is not possible, but that chicks responded so quickly under *ad libitum* conditions suggests that there may be threshold differences between chicks and rats. That both chicks and rats increase their food intake suggests that βCT's orexigenic effect was most likely