Neuroscience Letters 651 (2017) 52–56

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

The central effects of alpha-melanocyte stimulating hormone (α-MSH) in chicks involve changes in gene expression of neuropeptide Y and other factors in distinct hypothalamic nuclei

Meghan S. Delp, Mark A. Cline, Elizabeth R. Gilbert*

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

HIGHLIGHTS

- The hypothalamic mechanisms that mediate the effects of α-MSH on food intake in birds are unclear.
- Central α-MSH increased chick c-Fos immunoreactivity in the ARC, DMN, LH, and PVN.
- α-MSH increased NPY and AgRP mRNA in the ARC and reduced NPYR1 mRNA in the PVN.
- In the DMN, NPY and DDC mRNAs increased in α-MSH-treated chicks.
- Results suggest that α-MSH treatment affects NPY/AgRP production and signaling.

ARTICLE INFO

Article history:
Received 21 March 2017
Received in revised form 21 April 2017
Accepted 28 April 2017
Available online 1 May 2017

Keywords:
α-MSH
Arcuate nucleus
C-Fos
Chick
Dorsomedial nucleus
Hypothalamus
Paraventricular nucleus
NPY
Satiety

ABSTRACT

Alpha-melanocyte stimulating hormone (α-MSH) is a satiety-inducing factor in birds and mammals although central mechanisms mediating its effects on appetite in birds are poorly understood. Thus, the objective of the present study was to determine effects of centrally-injected α-MSH on c-Fos and gene expression in chick appetite-associated hypothalamic nuclei. At 4 days post-hatch, 3 h-fasted chicks were intracerebroventricularly (ICV) injected with 0 (vehicle) or 0.12 nmol α-MSH and 1 h later, hypothalamus samples were collected for measuring c-Fos immunoreactivity and mRNA abundance of appetite-associated factors in hypothalamic nuclei. There were more c-Fos immunoreactive cells in the arcuate nucleus (ARC), dorsomedial nucleus (DMN), lateral hypothalamus (LH), and paraventricular nucleus (PVN) of α-MSH- than vehicle-injected chicks. Neuropeptide Y (NPY), oxytocin receptor (OXTR), and agouti-related peptide (AgRP) mRNAs were greater in α-MSH- than vehicle-injected chicks in the ARC. In the PVN, NPY receptor sub-type 1 (NPYR1) mRNA was reduced while c-Fos mRNA was increased in response to treatment with α-MSH. NPY, c-Fos, and DOPA decarboxylase (DDC) mRNAs were greater in treated than vehicle-injected chicks in the DMN. Results suggest that during the first hour post-injection, the appetite-inhibiting effects of α-MSH involve activation of the ARC, DMN, PVN, and LH, and corresponding changes in transcriptional regulation of factors involved with NPY, AgRP and mesotocin signaling, and monoamine synthesis. The effects of these changes may include an inhibition of NPY signaling in the PVN to induce satiety and stimulation of NPY/AgRP neurons in the ARC in an attempt to restore homeostatic levels of food intake.

1. Introduction

The melanocortin system plays an important role in appetite regulation and is comprised of pro-opiomelanocortin (POMC)-derived peptides, melanocortin receptors (MCRs) 1 through 5, agouti and agouti-related peptide (endogenous MCR antagonists), and melanocortin 2 receptor accessory proteins (MRAPs) [1,2]. The hypothalamic melanocortin system consists of POMC/cocaine-amphetamine regulated transcript (CART) neurons and Neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons in the arcuate nucleus that project to second-order neurons that express receptors for their products [3]. The POMC-derived peptides are a product of post-translational cleavage and include adrenocorticotropic hormone (ACTH), β-endorphin, and α, β, and γ-MSH, among others [2].

* Corresponding author.
E-mail address: egilbert@vt.edu (E.R. Gilbert).

http://dx.doi.org/10.1016/j.neulet.2017.04.064
0304-3940/

Alpha-MSH inhibits feeding in rodents via MCRs 3 and 4, which are widely distributed in hypothalamic nuclei [4]. When α-MSH was applied to the third ventricle of rats for 6 consecutive days, food intake and body weight were reduced and there was increased c-Fos immunoreactivity in the paraventricular nucleus (PVN) of the hypothalamus [5].

Central injection of α-MSH also potently inhibits food intake in birds [6,7]. In broiler chicks, central injection of 0.12 nmol α-MSH reduced food intake, with treated chicks having consumed about a fourth of the amount of food as vehicle-injected chicks at 1 h post-injection [8]. Similarly, 40 and 400 pmol doses of α-MSH reduced food intake in 4 day-old broiler chicks within 30 min post-injection [9]. Although multiple groups have reported on the potent anorexigenic effects of α-MSH in birds, the molecular mechanisms underlying central effects of α-MSH on hypothalamic function are unclear. Hence, the objective of this experiment was to determine the effects of centrally administered α-MSH on hypothalamic c-Fos and mRNA abundance in specific nuclei. To our knowledge, this is the first report of the distribution of appetite-associated factor mRNA in different hypothalamic nuclei in an avian species after α-MSH injection.

2. Materials and methods

2.1. Animals

Cobb-500 chicks were obtained from a commercial hatchery on the morning of the hatch from parental stocks that were 30–34 weeks of age. On day 1 post-hatch, they were caged individually in an environmentally controlled room at 30 ± 2 °C and 50 ± 5% relative humidity with *ad libitum* access to a corn and soybean meal-based mash diet (22% crude protein and 3000 kcal ME/kg) and tap water. The composition of the diets is reported elsewhere [10]. For each experiment, chicks were randomly selected to receive injection of either vehicle (avian artificial cerebrospinal fluid) or 0.12 nmol α-MSH, using a randomized complete block design with body weight as the blocking factor, and injections were performed on the morning of day 4 post-hatch. The dose selected and timing of sample collection were based on our previous studies with the intention of evaluating effects during robust neuronal activation in the hypothalamus and reduced food intake [6,8]. Chicks within each experiment were from the same hatch, and each experiment was conducted between 08.00 and 13.00 h. 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 Tech Institutional Animal Care and Use Committee.

2.2. Intracerebroventricular (ICV) injection procedure

Chicks were injected using an adapted method [11] that does not appear to induce physiological stress [12]. 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. The α-MSH (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 location. The sequence of α-MSH (SYSMEHFRWGKPV) is identical to chicken [7]. After data collection, each chick was decapitated and its head sectioned along the frontal plane to determine the site of injection. Any chick without dye present in the lateral ventricle system was eliminated from analysis. The sex was identified via dissection. Numbers of chicks within each group for an experiment are presented in the figure captions.

2.3. Experiment 1: hypothalamic c-Fos immunohistochemistry

After a 3 h fast with access to water, injections were performed, and at 1 h post-injection, chicks were deeply anesthetized with sodium pentobarbital via cardiopuncture, then perfused via the carotid artery with ice-cold 0.9% NaCl followed by 4% paraformaldehyde in 0.1% M phosphate buffer (PB) containing 0.2% picric acid at pH 7.4. Brains were removed from the skulls and post-fixed for 1 h in the same solution, after which they were blocked through a series of sucrose incubations, consisting of 20% and 30% in 0.1 M PB until they sank. Several 40 μm coronal sections that contained appetite-related nuclei were collected in 0.02 M PB saline (PBS) containing 0.1% sodium azide using a cryostat at −15 °C. The arcuate nucleus (ARC), ventromedial hypothalamus (VMN), dorsomedial nucleus (DMN), paraventricular nucleus (PVN), and lateral hypothalamic area (LH) were collected and immediately processed.

Free-floating sections were pre-blocked for 1 h with 10% normal goat serum (NGS) and 0.3% Triton X-100 in 0.02 M PBS. To inhibit endogenous peroxidase activity, sections were incubated in 1.5% hydrogen peroxide and 50% methanol in deionized water for 30 min. Following a 3 × 10 min wash in buffer (0.05% NGS and 0.3% Triton X-100 in 0.02 M PBS), sections were incubated with rabbit polyclonal anti-c-Fos at a dilution of 1:20,000 in PBS containing 0.3% Triton X-100, 1% NGS, and 1% blocking reagent for 48 h under slow oscillation at 4 °C. For assay controls, the primary antibody was substituted with normal rabbit serum. Sections were then rinsed 3 × 10 min in wash buffer and incubated with biotinylated goat anti-rabbit secondary antibody at a dilution of 1:200 in PBS containing 1% NGS for 2 h at room temperature. Following a rinse with PBS, sections were processed with avidin-biotin horseradish peroxidase complex at a dilution of 1:200. Reactions were visualized with the DAB Substrate kit for Peroxidase (Vector Laboratories) for 10 s, mounted on gelatin-coated slides and cover-slipped with VectaMount (Vector Laboratories). Anatomy was confirmed and a digital micrograph captured for each section. Overlays containing the respective nuclei boundaries were digitally merged with micrographs and the number of c-Fos immunoreactive cells within each respective nucleus counted by a technician blind to treatment. Data were analyzed by Student's *t*-tests (control vs. treated within each nucleus) with significance set at $P < 0.05$.

2.4. Experiment 2: mRNA abundance in hypothalamic nuclei

After a 3 h fast with access to water, injections were performed, and at 1 h post-injection, each chick was deeply anesthetized with sodium pentobarbital via cardiopuncture, then perfused via the carotid artery with 2.5 mL of RNA stabilizing buffer (16.7 mM sodium citrate, 13.3 mM EDTA, and 3.5 M ammonium sulfate; pH = 5.2). Within 30 min of perfusion, brains were sectioned in a cryostat at −10 °C into 500 μm thick coronal sections. Sections were collected from the rostral to caudal direction: the LH and PVN corresponding to 8.0 and 7.4 from interaural, respectively, and the DMN and ARC (also lateral to infundibular nucleus) corresponding to 5.4 interaural based on published anatomy [13].

We selected to isolate nuclei for total RNA isolation that responded to treatment in Experiment 1 with increased c-Fos immunoreactivity. Nuclei biopsies were collected on a metal block housed on dry ice, using sterile disposable biopsy instruments (1 mm, Braintree Scientific Inc., Braintree, MA). The biopsies were immediately submerged in RNA lysis buffer with 1% beta-