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Satiety induced by central stresscopin is mediated by corticotrophin-releasing factor receptors and hypothalamic changes in chicks

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ABSTRACT

The central mechanism that mediates stresscopin (SCP)-induced satiety is poorly understood, and its effect on avian appetite is not documented. Thus, this study was conducted to elucidate some of the central and behavioral mechanisms that are associated with SCP-induced satiety using broiler- and layer-type chicks (*Gallus gallus*) as model organisms. In Experiment 1, broiler-type chicks responded with decreased food and water intake but had increased plasma corticosterone concentration after intracerebroventricular (ICV) SCP injection. However, the effect on water intake was secondary to food intake, since food-restricted SCP-treated broiler-type chicks did not reduce water intake in Experiment 2. In Experiment 3, layer-type chicks responded with decreased food intake at much lower doses than broiler-type chicks. In Experiment 4, astressin (a non-selective corticotrophin-releasing factor [CRF] receptor antagonist) prevented SCP-induced anorexia in broiler-type chicks. In Experiment 5, SCP-treated broiler-type chicks had an increased number of c-Fos immunoreactive cells in the ventromedial hypothalamus, parvicelluar and magnocellular divisions of the paraventricular nucleus and the periventricular nucleus. In Experiment 6, SCP-treated broiler-type chicks had decreased feeding pecks and increased jumping, distance moved and more escape attempts. Thus, we conclude that central SCP causes anorexigenic and other behavioral effects in chicks, and the hypothalamus and CRF receptors are involved.

1. Introduction

The 41 residue corticotrophin-releasing factor (CRF) is an adrenocorticotrophin (ACTH) secretagogue which initiates an organism's response to a stressor (Vale et al., 1981). While the major biological function of CRF is modulation of stress coping mechanisms, it also is one of the most potent inhibitors of ingestion in mammals (Richard et al., 1996, 2002; Rothwell, 1990) and avians (Denbow et al., 1999; Zhang et al., 2001).

CRF binds to at least two major G protein-coupled receptors, CRF_1 and CRF_2. CRF_1 is primarily associated with ACTH released in response to stressors (Smith et al., 1998; Timpl et al., 1998) and is also associated with short-term anorexia (Hotta et al., 1999; Reyes et al., 2001). Coste et al. (2001) and Hashimoto et al. (2001) provided evidence that stimulation of CRF_2 mediates stress coping mechanisms; however, Heinrichs et al. (1997) show that CRF_2 is associated with anxiogenic-like behavior. Activation of CRF_2 causes a potent decrease in food intake (Hotta et al., 1999), and is associated with starvation (Nazarloo et al., 2002). CRF_2 is located in the hypothalamus near satiety-related nuclei (Lovenberg et al., 1995) and in the chick diencephalon (De Groef et al., 2004), whereas CRF_1 is located more in the cortex and pituitary (Potter et al., 1994). According to Reyes et al. (2001) CRF_2 is responsible for long-term anorexia associated with CRF. Additionally, deletion of CRF_2 in mice causes anxiety-associated behavior (Bale et al., 2000) and increased corticosterone concentration following short-term restraint (Coste et al., 2000).

The urocortins, a family of CRF-like peptides, bind to and activate CRF receptors. Members of this family include urocortin 1 (Vaughan et al., 1995), stresscopin-related peptide (Reyes et al., 2001) and stresscopin (SCP, Lewis et al., 2001). Stresscopin-related peptide and SCP are selective agonists for CRF_2 (Hsu and Hsueh, 2001) and cause reduced food intake in rats (Reyes et al., 2001; Ohata and Shibasaki, 2004). Stresscopin-related peptide and SCP have N-terminally shortened analogs, urocortin 2 and urocortin 3, which are all hypothetical endogenous ligands for the CRF_2 receptor. These analogs were identified by two independent research groups (Hsu and Hsueh, 2001; Lewis et al., 2001) from sequence homology searches of the mouse and human genomes. Human stresscopin encodes a preproprotein of 161 amino acids and a putative mature protein of 40 amino acids, whereas the 112-amino-acid open reading frame of stresscopin-related peptide contains a predicted 43-amino-acid mature peptide (Hsu and Hsueh, 2001). Urocortin 3 prohormone sequences from several species (including chicken) contain protypical dibasic cleavage site (arginine-arginine) but is not conserved in humans and rodents

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and cleaved following the threonine-lysine residues. Zhang et al. (2001) showed that urocortin 1 causes reduced feeding in chicks, but to our knowledge the effects of stresscopin-related peptide and SCP have not been studied within the avian class.

Therefore, the purpose of the present study was to examine some appetite-associated responses after central injection of SCP, and to use SCP to elucidate the contribution of CRF_2 to the regulation of satiety in the chick. We measured food and water intake, plasma corticosterone concentration, and behaviors after central SCP injection. Additionally, the involvement of CRF receptors in SCP-induced anorexia was tested.

2. Methods

2.1. Animals

Day of hatch unsexed Cobb-500 (a broiler type, primarily raised for consumption by humans) and White Leghorn (a layer type, primarily used for egg consumption by humans) chicks (*Gallus gallus*) were obtained from a commercial hatchery. In each experiment chicks were from separate hatches. They were caged individually in a room at 30 ± 2 °C and 50 ± 5% relative humidity with *ad libitum* access to a mash diet (20% crude protein, 2685 kcal metabolizable energy/kg) and water. All trials were conducted 4 days post hatch unless otherwise noted. 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 Radford University or Ehime University Institutional Animal Care and Use Committee.

2.2. Intracerebroventricular (ICV) injection procedure

Chicks were injected using a method adapted from Davis et al. (1979). 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 in the un-anaesthetized chick for 10 s post injection to reduce backflow. Chicks were randomly assigned to treatments. SCP (4367.2 MW; American Peptide Co., Sunnyvale, CA, USA) was dissolved in artificial cerebrospinal fluid (aCSF; Anderson and Heisey, 1972) as a vehicle for a total injection volume of 5 µL with 0.1% Evans Blue dye to facilitate injection site localization. After data collection, the chick was decapitated and its head sectioned along the frontal plane to determine site of injection. Any chick without dye present in the lateral ventricle system was eliminated from analysis. After decapitation, sex was visually determined by dissection.

2.3. Experiment 1: food and water intake of broiler-type chicks

Broiler-type chicks, fasted for 180 min (to intensify hunger), were randomly assigned to receive either 0 (vehicle only), 92 (0.4 µg), 184 (0.8 µg) or 368 (1.6 µg) pmol SCP by ICV injection. After injection, chicks were returned to their individual cages and given *ad libitum* access to both food and water. Food and water intake were monitored (0.01 g) every 30 min for 180 min post injection. Water weight (g) was converted to volume (ml; 1 g = 1 ml). For this experiment only, two trials were conducted 2 wk apart (to increase the *n* for the purpose of testing for a sex effect), and a trial effect was not detected. Thus, data from both trials were pooled. Food containers were filled to one quarter capacity to reduce spillage. Chicks were decapitated 180 min after injection and trunk blood was collected into microcentrifuge tubes containing 0.06 mg ethylenediaminetetraacetic acid. Microcentrifuge tubes were immediately centrifuged at 3000 ×*g* for 10 min and the supernatant was collected and stored at −80 °C until assay. Plasma corticosterone concentrations were determined in duplicate using a commercially available enzyme immunoassay kit (Correlate-EIA; Assay Designs Inc., Ann Arbor, MI, USA). Data were analyzed using analysis of variance (ANOVA) at each time point using the GLM procedure of SAS. The model included SCP dose, sex and the interaction of sex with SCP dose. Sex and the interaction of sex and SCP dose were not significant and were eliminated from the model (and the effect of sex was not tested in proceeding experiments). If significant treatment effects were found, Tukey's method of multiple comparisons was used to separate the means at each time period. In this experiment 14 to 18 chicks per SCP dose were available for statistical analysis. For this and all proceeding experiments, statistical significance was set at $P<0.05$.

2.4. Experiment 2: effect on water intake without feeding of broiler-type chicks

The experimental procedures were identical to those in Experiment 1 except food was withheld during the observation period. Broiler-type chicks were fasted prior to injection to mimic the conditions of Experiment 1. Blood was not collected for plasma corticosterone determination. For this experiment 9 to 10 chicks per SCP dose were available for statistical analysis.

2.5. Experiment 3: effect on food intake of layer-type chicks

The experimental procedures were identical to those in Experiment 1 except 5 day post hatch layer-type chicks were randomly assigned to receive either 0, 92, or 184 pmol SCP by ICV injection. Blood was not collected for plasma corticosterone determination. For this experiment 6 to 8 chicks per SCP dose were available for statistical analysis.

2.6. Experiment 4: CRF receptor blockade of broiler-type chicks

The experimental procedures were identical to those in Experiment 1 except that broiler-type chicks were randomly assigned to receive either vehicle only, 184 pmol SCP, 6 nmol astressin (3563.3 MW; American Peptide Co., Sunnyvale, CA, USA), or 184 pmol SCP + 6 nmol astressin by ICV injection. Blood was not collected for plasma corticosterone determination. The dose of astressin was based on Saito et al. (2005) and Tachibana et al. (2006) in chicks. In this experiment 5 to 7 chicks per SCP dose were available for statistical analysis.

2.7. Experiment 5: hypothalamic c-Fos immunoreactive cell counts of broiler-type chicks

Broiler-type chicks, fasted for 180 min, were randomly assigned to receive either vehicle only or 184 pmol SCP ICV, and were immediately given *ad libitum* access to both food and water post injection. Food and water were given to mimic the conditions of Experiment 1. Thirty minutes after central injection chicks were deeply anesthetized with an IP injection of sodium pentobarbital (30 mg/kg body weight) and then decapitated. The brain was immediately fixed with a 2% paraformaldehyde 0.1% glutaraldehyde solution via the left carotid artery. The head was positioned in a stereotaxic instrument and the brain sectioned frontally according to Puelles et al. (2007). The blocked brain was placed in 30% sucrose in phosphate buffered saline for 48 h at 4 °C. Using a cryostat, sections 40 µm thick were cut from areas of the brain that contained the arcuate nucleus (ARC), dorsomedial nucleus (DMN), lateral hypothalamus (LH), parvicelluar division of the paraventricular nucleus (PaPC), magnocellular division of the paraventricular nucleus (PaMC), superchiasmatic nucleus (SCh), periventricular nucleus (PHN) and the ventromedial hypothalamus (VMH). Sections were incubated with anti-Fos polyclonal antibody (1:600, v/v;