Pharmacology, Biochemistry and Behavior 92 (2009) 663–669

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

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

Mark A. Cline [a,*], Brian C. Prall [a], J. Orion Rogers [a], Tetsuya Tachibana [b]

[a] Department of Biology, Radford University, Radford, Virginia 24142, USA
[b] Laboratory of Animal Production, Department of Agrobiological Science, Faculty of Agriculture, Ehime University, Matsuyama 790-8566, Japan

ARTICLE INFO

Article history:
Received 1 December 2008
Received in revised form 10 March 2009
Accepted 17 March 2009
Available online 24 March 2009

Keywords:
Appetite
Chick
CRF
Hypothalamus
Paraventricular nucleus
Stresscopin

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

* Corresponding author. PO Box 6931, Radford, VA 24142, USA.
E-mail address: mcline@radford.edu (M.A. Cline).

0091-3057/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2009.03.005

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;

Sigma, St. Louis, MO, USA; significant homology with chicken c-Fos) for 48 h at 4 °C and then with an alkaline phosphatase-conjugated secondary monoclonal antibody (1:600 v/v; Sigma) at room temperature for 2 h. The secondary antibody was visualized using alkaline phosphatase substrate kit III (Vector Laboratories Ltd., Burlingame, CA). The number of reactive cells was counted from the injected side of the brain (left) in an area 0.2 mm^2 located in the center of the respective nucleus using light microscopy by a single technician blind to treatment, according to coordinates based on Puelles et al. (2007). Two sections were counted and averaged to arrive at the value for each chick. Data were analyzed by ANOVA for the effect of SCP using the GLM procedure of SAS. Six vehicle only and 4 SCP-treated chicks were available for statistical analysis.

2.8. Experiment 6: behavior of broiler-type chicks

Broiler-type chicks were kept in individual cages with auditory but not visual contact with each other (to reduce isolation stress during behavior measurement), and were randomly assigned to receive either vehicle only or 184 pmol SCP ICV. Following a 3 h fast, ICV injections were made and chicks were immediately placed in a 290×290 mm acrylic recording arena with food and water containers in diagonal corners. Chicks were simultaneously and automatically recorded from 3 angles for 30 min post injection on DVD and data were analyzed in 300 s intervals using ANY-maze behavioral analysis software (Stoelting, Wood Dale, IL, USA). Locomotion (m travelled), the amount of time spent standing, sitting, perching, preening, or in deep rest, and the number of jumps, food and exploratory pecks, and drinks, were quantified. Food pecks were defined as pecks within the food container, whereas any other pecks were counted 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. Data were analyzed by the Mann–Whitney *U* test (due to non-heterogeneous variance). In this experiment 9 chicks per treatment were available for statistical analysis.

3. Results

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

Stresscopin administered ICV caused reduced food intake (Fig. 1). Broiler-type chicks treated with 92 pmol SCP had similar food intake to vehicle-treated chicks. However, food intake in chicks that received 184 and 368 pmol SCP was reduced compared to control. Both groups of 184 and 368 pmol SCP injected chicks had a similar reduction in food intake from 30 to 120 min post injection. At 150 and 180 min chicks treated with 368 pmol consumed less food than those treated with 184 pmol SCP.

Water intake was also reduced by SCP injection (Fig. 2). Broiler-type chicks treated with 92 pmol SCP did not reduce water intake. However, chicks treated with 184 and 368 pmol SCP reduced water intake. The group injected with 184 pmol SCP had reduced water intake at 90 and 120 min post injection, and this effect was not significant by 150 min post injection and thereafter. The group injected with 368 pmol SCP was the only group to have reduced water intake at all observation times.

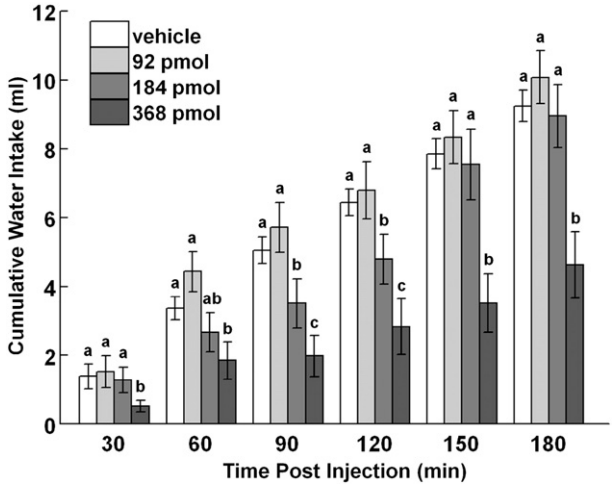

Fig. 2. Cumulative water intake following ICV injection of increasing doses of SCP in broiler-type chicks (Experiment 1). Values are mean ± standard error; bars with different letters are different from each other within a time point ($P<0.05$).

3.2. Experiment 1: plasma corticosterone concentration of broiler-type chicks

The reduction in feeding measured in Experiment 1 was accompanied by increased plasma corticosterone concentration (Fig. 3). Broiler-type chicks treated with the highest dose of SCP, 368 pmol, had a two fold increase in plasma corticosterone

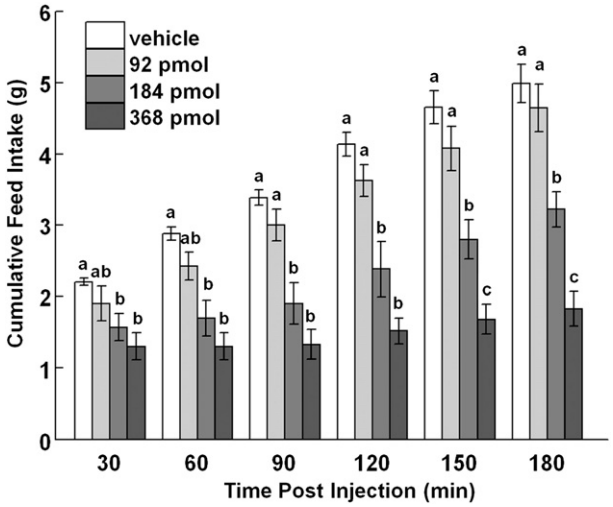

Fig. 1. Cumulative food intake following ICV injection of increasing doses of SCP in broiler-type chicks (Experiment 1). Values are mean ± standard error; bars with different letters are different from each other within a time point ($P<0.05$).

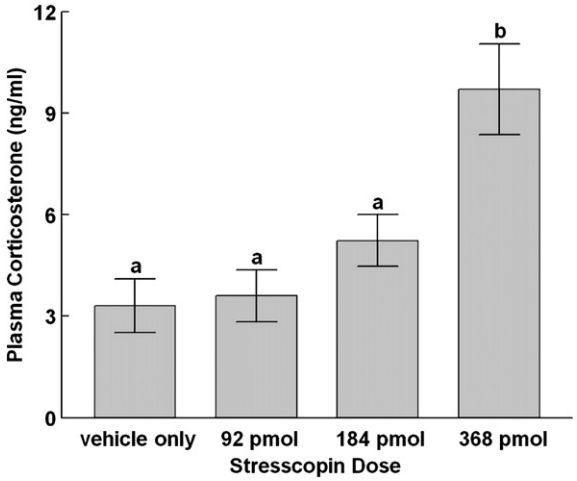

Fig. 3. Plasma corticosterone concentration at 180 min following ICV injection increases as SCP increases in broiler-type (Experiment 1). Values are mean ± standard error; bars with different letters are different from each other ($P<0.05$).

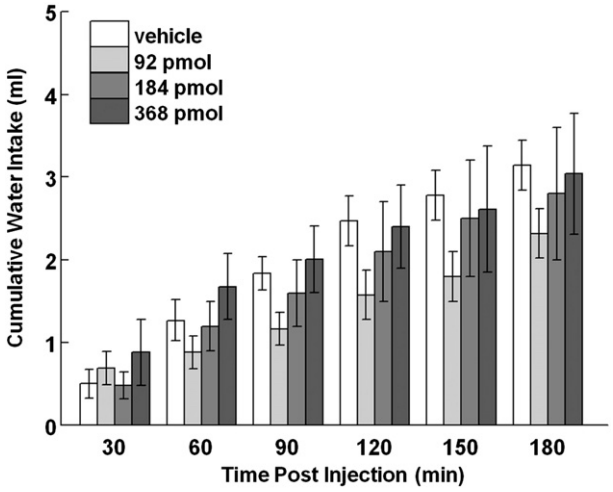

Fig. 4. Cumulative water intake following ICV injection of SCP in food-restricted broiler-type chicks (Experiment 2). Values are mean ± standard error.

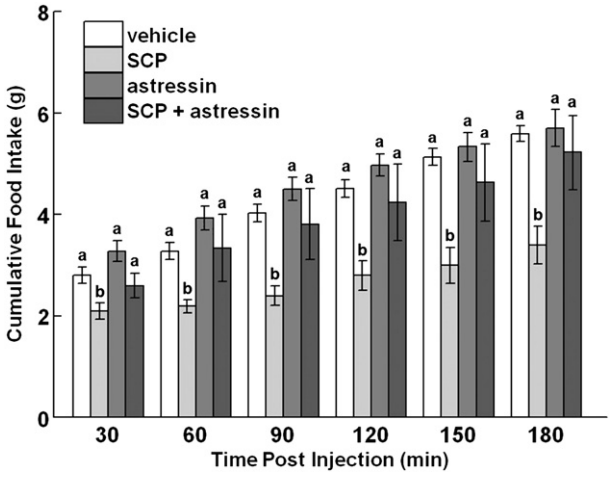

Fig. 6. Cumulative food intake following blockade of CRF receptors and ICV injection of 184 pmol SCP in broiler-type chicks (Experiment 4). Values are mean ± standard error; bars with different letters are different from each other ($P<0.05$).

concentration 180 min post injection. The intra-assay precision was 8.9%.

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

To ascertain if the reduction in water intake was secondary to reduced food intake, water intake was measured after SCP injection in food-restricted broiler-type chicks. When food was not available, SCP-treated chicks did not have reduced water intake as compared to vehicle-treated chicks (Fig. 4).

3.4. Experiment 3: food intake of layer-type chicks

SCP administered ICV caused reduced food intake at all times in layer-type chicks (Fig. 5). Layer-type chicks treated with 92 and 184 pmol SCP had reduced food intake compared to vehicle-treated chicks. Both groups of chicks that received of 92 and 184 pmol SCP had a similar reduction in food intake, and compensatory food intake was not observed in either of these groups following injection.

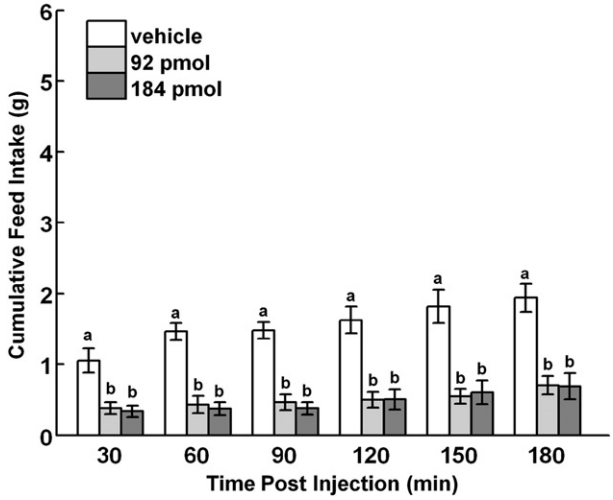

Fig. 5. Cumulative food intake following ICV injection of SCP in food-restricted layer-type chicks (Experiment 3). Values are mean ± standard error.

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

In Experiment 4, broiler-type chicks treated with SCP had reduced food intake at all observation times (Fig. 6). Chicks that received either astressin or the dual injection of SCP and astressin did not exhibit reduced food intake at any observation time.

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

In Experiment 5, broiler-type chicks that received ICV SCP had an increased number of c-Fos immunoreactive cells in the VMH, PaPC,

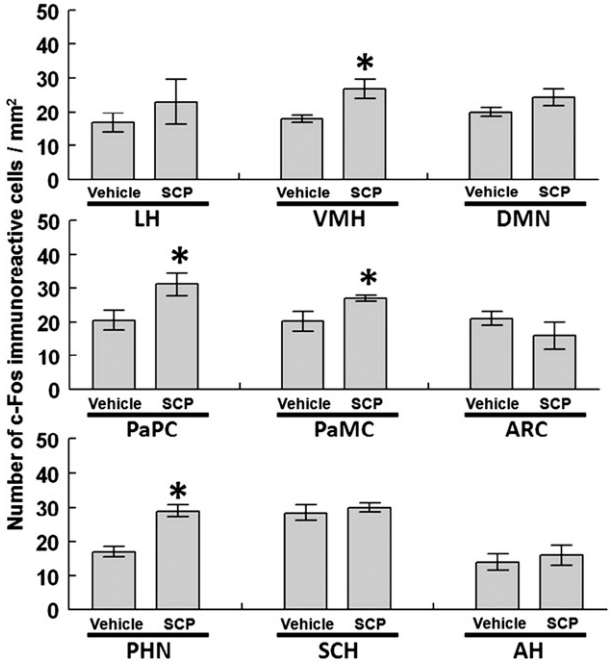

Fig. 7. Effect of 184 pmol SCP on the number of c-Fos immunoreactive cells in the anterior hypothalamus (AH), arcuate nucleus (ARC), dorsomedial nucleus (DMN), lateral hypothalamus (LH), magnocellular division of the paraventricular nucleus (PaMC), parvicelluar division of the paraventricular nucleus (PaPC), periventricular nucleus (PHN), superchiasmatic nucleus (SCh), and the ventromedial hypothalamus (VMH) in broiler-type chicks (Experiment 3). (*) denotes difference from control ($P<0.05$). Values are mean ± S.E.M.

Table 1
Mean ± standard error of count type behaviors following ICV injection of vehicle or of 184 pmol SCP in broiler-type chicks (Experiment 6).

Time post injection (s)							
Behavior	Treatment	300	600	900	1200	1500	1800
Feeding pecks	Control	204 ± 77	518 ± 107	813 ± 163	1008 ± 155	1273 ± 175	1339 ± 173
	SCP	239 ± 71	455 ± 120	522 ± 144	564 ± 162	594 ± 171*	643 ± 171*
Exploratory pecks	Control	6.8 ± 6.7	13.1 ± 7.9	17.4 ± 8.0	19.2 ± 8.0	26.7 ± 6.5	24.9 ± 9.4
	SCP	0.7 ± 0.4	4.4 ± 1.5	11.1 ± 3.0	16.8 ± 3.8	24.2 ± 9.3	35.1 ± 8.0
Jump	Control	0.3 ± 0.3	0.7 ± 0.5	0.7 ± 0.5	0.7 ± .05	0.8 ± 0.5	1.1 ± 0.6
	SCP	0.4 ± 0.2	1.2 ± 0.5	4.2 ± 1.3*	6.1 ± 2.1*	7.4 ± 2.3*	7.9 ± 2.4*
Distance moved (m)	Control	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	0.8 ± 0.3	1.0 ± 0.4	1.4 ± 0.6
	SCP	0.3 ± 0.1	1.1 ± 0.3	2.5 ± 0.5*	3.8 ± 0.6*	5.27 ± 0.8*	6.3 ± 1.0*
Escape attempts	Control	0.1 ± .01	0.4 ± 0.3	0.8 ± 0.5	1.0 ± 0.8	1.3 ± 0.8	1.8 ± 1.1
	SCP	0.1 ± .01	0.8 ± 0.8	1.9 ± 0.8	2.4 ± 1.0	3.4 ± 1.0*	3.9 ± 1.3

* denotes different from control ($P<0.05$).

PaMC and PHN with 51%, 52%, 34% and 71% increased over vehicle-treated chicks respectively (Fig. 7). SCP treatment did not affect the number of c-Fos immunoreactive cells in the LH, DMN, ARC, SCH or AH.

3.7. Experiment 6: behavior of broiler-type chicks

Broiler-type chicks that received ICV SCP had a decreased number of feeding pecks 1500 s post injection and thereafter (Table 1). However, the number of exploratory pecks was not affected by the treatment. At 900 s and thereafter ICV SCP-treated chicks jumped and moved more. Those chicks treated with SCP also attempted escape more frequently, an effect that was significant at 1500 s post injection.

Most timed-type behaviors were not affected by ICV SCP including stand, sit, and preen time (Table 2). However, none of the SCP-treated chicks exhibited deep rest at or before 1500 s post injection, and at 1800 s post injection deep rest was decreased in SCP-treated chicks.

4. Discussion

SCP is the latest member of the CRF family to be identified (Hsu and Hsueh, 2001; Lewis et al., 2001) in a range of mammalian species. We were interested in the central effects of SCP since it is located in greatest abundance in the hypothalamus, near satiety circuitry (Li et al., 2002) in proximity to areas of CRF_2 expression (Lovenberg et al., 1995). The human SCP we injected (TKFTLSLDVPTNIMNLLFNIAKAKNLRAQAAANAHLMAQI) has 92% sequence identity to that reported for chicken (TKVTLSLDVPTNIMNILFNIAKAKNLRAKAAANAHLMAQI, accession CGNC:16844). In chicken, the SCP gene is located on chromosome 1. Using the chick we have presented results that may be interpreted as central SCP causes anorexigenic effects that are mediated at the hypothalamus through interactions with the hypothalamic–pituitary–adrenal (HPA) axis.

In Experiment 1, a dose as low as 184 pmol of SCP ICV was effective at reducing food intake in broiler-type chicks that had been fasted for 3 h. In rats fasted for 20 h, 3000 pmol SCP ICV was necessary to cause reduced food intake, and this effect was only detected at 30 min post injection (Fedeli et al., 2006). Thus, in broiler-type chicks the threshold for SCP-induced anorexia is approximately 16 times less than in rats, but the difference in fasting times should be considered. However, when Fekete et al. (2007) did not fast rats and injected urocortin 3 (an independent analog of SCP that is also a putative ligand for CRF_2 receptors) at similar doses, a similar magnitude of anorexia was measured as what occurred in the present study. Broiler-type chicks have been selected for accelerated growth which causes them to eat more (but which are not hyperphagic in the sense that they become obese) than layer-type chicks that have been selected for egg production (National Research Council, 1994; Taylor and Field, 2008). The former has a body weight fivefold higher than of the latter at 6 weeks of age (Zhao et al., 2004). Experiment 3 was conducted because the increased food intake and accelerated body weight gain of broiler-type chicks are likely associated with selection for less potent anorexigenic neuropeptide systems. This thesis was supported since layer-type chicks responded with anorexia to SCP at a much lower dose than did broiler-type chicks. Thus, the threshold of anorexigenic response is lower in layer- than broiler-type chicks. In both types of chicks, the magnitude of divergence of effective doses of SCP from vehicle-treated chicks increased as time progressed. This may be interpreted as SCP induces sustained anorexia in chicks and that compensatory food intake does not occur soon after SCP injection.

In the present study, the magnitude of anti-dipsogenic response was greater than the anorexigenic response for broiler-type chicks treated with 368 pmol (2.7 vs. 1.6 orders less, respectively). We speculated that the effect on water intake may be secondary to that of food intake; this thesis was supported by the results of Experiment 2. Thus, the decreased water intake observed in Experiment 1 is decreased prandial water intake. The lack of an effect on water intake in food-restricted broiler-type chicks may also be interpreted as the anorexigenic effect observed in Experiment 1 is not due to malaise. It is typical for broiler-type chicks to consume much more water than food after undergoing a 180 min fast (Cline et al., 2008a, 2009).

Several peptides that affect appetite do so either directly or partly by the activation of CRF receptors. This is the case in chicks for other

Table 2
Mean ± standard error of timed-type behaviors following ICV injection of vehicle or 184 pmol SCP in broiler-type chicks (Experiment 6).

Time post injection (s)							
Behavior	Treatment	300	600	900	1200	1500	1800
Stand time (s)	Control	284.7 ± 14.0	545.0 ± 26.0	774.3 ± 53.9	966.0 ± 83.7	1139.3 ± 120.9	1288.6 ± 159.6
	SCP	292.6 ± 5.2	545.4 ± 37.4	800.7 ± 69.1	1041.8 ± 89.8	1304.1 ± 97.4	1546.0 ± 98.2
Sit time (s)	Control	14.4 ± 13.5	43.2 ± 20.1	100.4 ± 42.7	193.4 ± 65.5	299.8 ± 98.1	401.8 ± 122.8
	SCP	5.2 ± 5.2	48.6 ± 43.2	86.4 ± 70.3	131.5 ± 93.0	154.3 ± 102.0	194.6 ± 103.7
Deep rest (s)	Control	7.8 ± 7.8	12.6 ± 12.6	12.6 ± 12.7	26.2 ± 20.4	40.6 ± 24.1	86.2 ± 41.3
	SCP	0	0	0	0	0	5.8 ± 5.8*
Preen time (s)	Control	0.3 ± 0.2	3.4 ± 3.1	11.8 ± 8.4	13.4 ± 8.6	16.7 ± 8.4	19.4 ± 8.9
	SCP	1.9 ± 1.6	5.7 ± 2.8	12.6 ± 5.4	26.5 ± 10.7	41.3 ± 16.7	53.1 ± 21.1

anorexigenic peptides including glucagon-like peptide-1 (Tachibana et al., 2006), alpha-melanocyte-stimulating hormone (Tachibana et al., 2007), ghrelin (Saito et al., 2005) and amylin (Cline et al., 2008b). Therefore, Experiment 4 was designed to test if SCP-induced anorexia was mediated via CRF receptors. This is likely the case since SCP-induced anorexia was prevented by astressin, a non-selective CRF receptor blocker.

Although the anorexigenic effects of SCP were previously reported for rats (Fedeli et al., 2006), activation of hypothalamic nuclei measured by c-Fos immunoreactivity has not been reported. That the VMH and paraventricular nucleus (PVN) were activated may be interpreted as the reduction in food intake observed in Experiment 1 is primarily due to a reduction in appetite. These nuclei are associated with satiety perception (Kirchgessner et al., 1988; Dakin et al., 2001; Kalra et al., 1999) and are sites where CRF receptor mRNA is expressed (Wong et al., 1994). Additionally, the PVN has a dense concentration of CRF receptors (Konishi et al., 2003). The PHN, which was also activated by ICV SCP in the present experiment, does not affect appetite per se, but does compensate to maintain normal regulation of appetite when the PVN is disrupted (Kirchgessner et al., 1988). The DMN and ARC were examined in the present study since they too are associated with direct and indirect satiety perceptions (Bernardis, 1975). The SCH and AH are in proximity to nuclei that regulate appetite and were also studied. SCP likely influences the perception of satiety without affecting perception of hunger since the LH, a nucleus associated with hunger perception (Brobeck, 1946; Anand and Brobeck, 1951), was not affected by the treatment. Increased activation of the PVN and VMH in the present study may be a primary effect, since direct injection into these nuclei in rats causes an anorexigenic effect (Fekete et al., 2007).

While the PaMC of the PVN is associated with appetite perception (O'Shea and Gundlach, 1995), the PaPC division is also a key site for behavioral stress responses (Swanson et al., 1986), and its activation in Experiment 5 may account for the results obtained in Experiment 6. Increased jumping, movement, escape attempts and decreased deep rest support that ICV SCP induced a stress-like state in the broiler-type chicks. Similar to our present report, Ohgushi et al. (2001) reported that chicks treated with ICV CRF had increased jumping and stepping. Since jumping, movement and attempting escape are incompatible with ingestion, one explanation of the results from Experiment 6 is that these behaviors may be competitive with appetite and contribute indirectly to the anorexigenic response. However, water intake was not affected by the treatment in Experiment 2 which may be interpreted as anxiety-related behaviors are secondary to decreased appetite. It is possible that increased perception of satiety and other behavior modifications are coinciding and that neither is secondary to the other.

We conclude that central injection of SCP causes anorexigenic and other behavior modifications in chicks. Dose-dependent decreased food intake associated with SCP coincides with increased plasma corticosterone concentration. SCP does not affect water intake directly, but rather decreases prandial drink. The effect on food intake is mediated via CRF receptors and the hypothalamic mechanism mediating SCP-induced anorexia includes activation of the VMH, PaPC, PaMC and PHN. Activation of the PaPC may be associated with increased anxiety-related behaviors observed after ICV SCP. Thus, SCP is a likely mediator of chick appetite through changes in hypothalamic chemistry.

Acknowledgements

This work was supported in part by a grant from the Virginia Academy of Sciences. The authors would also like to thank anonymous reviewers for their significant contributions to this manuscript.

References

Anand BK, Brobeck JR. Hypothalamic control of food intake in rats and cats. Yale J Biol Med 1951;24:123–46.
Anderson DK, Heisey SR. Clearance of molecules from cerebrospinal fluid in chickens. Am J Physiol 1972;222:645–8.
Bale TL, Contarino A, Smith GW, Chan R, Gold LH, Sawchenko PE, et al. Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. Nat Genet 2000;24:410–4.
Bernardis LL. The dorsomedial hypothalamic nucleus in autonomic and neuroendocrine homeostasis. Can J Neurol Sci 1975;2:45–60.
Brobeck JR. Mechanism of the development of obesity in animals with hypothalamic lesions. Physiol Rev 1946;26:541.
Cline MA, Fouce D, Prall B. Central and peripheral alytesin cause short-term anorexigenic effects in neonatal chicks. Neuropeptides 2008a;42:283–91.
Cline MA, Nandar W, Smith ML, Pittman BH, Kelly M, Rogers JO. Amylin causes anorexigenic effects via the hypothalamus and brain stem in chicks. Regul Pept 2008b;146:140–6.
Cline MA, Calchary W, Nandar W. Effect of calcitonin gene-related peptide (CGRP) on avian appetite-related processes. Behav Brain Res 2009;196:242–7.
Coste SC, Kesterson RA, Heldwein KA, Stevens SL, Heard AD, Hollis JH, et al. Abnormal adaptations to stress and impaired cardiovascular function in mice lacking corticotropin-releasing hormone receptor-2. Nat Genet 2000;24:403–9.
Coste SC, Murray SE, Stenzel-Poore MP. Animal models of CRH excess and CRH receptor deficiency display altered adaptations to stress. Peptides 2001;22:733–41.
Dakin CL, Gunn I, Small CJ, Edwards CM, Hay DL, Smith DM, et al. Oxyntomodulin inhibits food intake in the rat. Endocrinology 2001;142:4244–50.
Davis JL, Masuoka DT, Gerbandt LK, Cherkin A. Autoradiographic distribution of l-proline in chicks after intracerebroventricular injection. Physiol Behav 1979;22:693–5.
De Groef B, Grommen S, Mertens I, Schoofs L, Kühn E, Darras V. Cloning and tissue distribution of the chicken type 2 corticotropin-releasing hormone receptor. Gen Comp Endocrinol 2004;138:89–95.
Denbow DM, Snapir N, Furuse M. Inhibition of food intake by CRF in chickens. Physiol Behav 1999;66:645–9.
Fedeli A, Policani F, Ubaldi M, Cippitelli A, Massi M, Ciccocioppo R. Activation of the nociceptin/orphanin FQ system is unable to reverse CRF2 receptor mediated anorexia in the rat. Peptides 2006;27:3284–91.
Fekete É, Inoue K, Zhao Y, Rivier JE, Vale WW, Szücs A, et al. Delayed satiety-like actions and altered feeding microstructure by a selective type 2 corticotropin-releasing factor agonist in rats: intra-hypothalamic urocortin 3 administration reduces food intake by prolonging the post-meal interva. Neuropsychopharmacology 2007;32:1052–68.
Hashimoto K, Makino S, Asaba K, Nishiyama M. Physiological roles of corticotropin-releasing hormone receptor type 2. J Endocrinol 2001;48:1–9.
Heinrichs S, Lapsansky J, Lovenberg T, De Souza E, Chalmers D. Corticotropin-releasing factor CRF1, but not CRF2, receptors mediate anxiogenic-like behavior. Regul Pept 1997;71:15–21.
Hotta M, Shibasaki T, Arai K, Demura H. Corticotropin-releasing factor receptor type 1 mediates emotional stress-induced inhibition of food intake and behavioral changes in rats. Brain Res 1999;823:221–5.
Hsu SY, Hsueh AJ. Human stresscopin and stresscopin-related peptide are selective ligands for the type 2 corticotropin-releasing hormone receptor. Nat Med 2001;7:605–11.
Kalra SP, Dube MG, Pu S, Xu B, Horvath TL, Kalra PS. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocr Rev 1999;20:68-100.
Kirchgessner AL, Sclafani A, Nilaver G. Histochemical identification of a PVN-hindbrain feeding pathway. Physiol Behav 1988;42:529–43.
Konishi S, Kasagi Y, Katsumata H, Minami S, Imaki T. Regulation of corticotropin-releasing factor (CRF) type-1 receptor gene expression by CRF in the hypothalamus. Endocr J 2003;50:21–36.
Lewis K, Li C, Perrin MH, Blount A, Kunitake K, Donaldson C, et al. Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. Proc Natl Acad Sci U S A 2001;98:7570–5.
Li C, Vaughan J, Sawchenko PE, Vale WW. Urocortin III-immunoreactive projections in rat brain: partial overlap with sites of type 2 corticotrophin-releasing factor receptor expression. J Neurosci 2002;22:991-1001.
Lovenberg TW, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, Desouza EB, et al. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. Proc Natl Acad Sci U S A 1995;92:836–40.
National Research Council. Nutrient Requirements of Poultry. 9th ed. Washington DC: National Academy Press; 1994.
Nazarloo HP, Nishiyama M, Tanaka Y, Asaba K, Hashimoto K. Down regulation of corticotropin-releasing hormone receptor type 2 mRNA in the rat cardiovascular system following food deprivation. Regul Pept 2002;105:121–9.
Ohata H , Shibasaki T. Effects of urocortin 2 and 3 on motor activity and food intake in rats. Peptides 2004;25:1703–9.
Ohgushi A, Bungo T, Shimojo M, Masuda Y, Denbow DM, Furuse M. Relationships between feeding and locomotion behaviors after central administration of CRF in chicks. Physiol Behav 2001;72:287–9.
O'Shea R, Gundlach L. Activity-linked alterations in cholecystokinin$_B$ receptor messenger RNA levels in magnocellular hypothalamic neurones by food and water deprivation in the rat. Neurosci Lett 1995;194:189–92.

Potter E, Sutton S, Donaldson C, Chen R, Perrin M, Lewis K, et al. Distribution of corticotropin-releasing factor receptor mRNA expression in the rat brain and pituitary. Proc Natl Acad Sci U S A 1994;91:8777–81.

Puelles L, Martinez-de-la-Torre M, Paxinos G, Watson C, Martinez S. The Chick Brain in Stereotaxic Coordinates: An Atlas Featuring Neurometric Subdivisions and Mammalian Homologies. 1st ed. Burlington: Academic Press; 2007.

Reyes TM, Lewis K, Perrin MH, Kunitake KS, Vaughan J, Arias CA, et al. Urocortin II: a member of the corticotropin-releasing factor (CRF) neuropeptide family that is selectively bound by type 2 CRF receptors. Proc Natl Acad Sci U S A 2001;98:2843–8.

Richard D, Rivest R, Naimi N, Timofeeva E, Rivest S. Expression of corticotrophin-releasing factor and it receptors in the brain of lean and obese Zucker rats. Endocrinology 1996;137:4786–95.

Richard D, Lin Q, Timofeeva E. The corticotrophin-releasing factor family of peptides and CRF receptors: their roles in the regulation of energy balance. Eur J Pharmacol 2002;440:189–97.

Rothwell N. Central effects of CRF on metabolism and energy balance. Neurosci Biobehav Rev 1990;14:263–71.

Saito E, Kaiya H, Tachibana T, Tomonaga S, Denbow M, Kangawa K, et al. Inhibitory effect of ghrelin on food intake is mediated by the corticotropin-releasing factor system in neonatal chicks. Regul Pept 2005;125:201–8.

Smith GW, Aubry JM, Dellu F, Contarino A, Gold LH, Chen R, et al. Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. Neuron 1998;20:1093–102.

Swanson LW, Sawchenko PE, Lind RW. Regulation of multiple peptides in CRF parvocellular neurosecretory neurons: implications for the stress response. Prog Brain Res 1986;68:169–90.

Tachibana T, Sato M, Oikawa D, Furuse M. Involvement of CRF on the anorexic effect of GLP-1 in layer chicks. Comp Biochem Physiol, Part A Mol Integr Physiol 2006;143:112–7.

Tachibana T, Oikawa D, Takahashi H, Boswell T, Furuse M. The anorexic effect of alpha-melanocyte-stimulating hormone is mediated by corticotrophin-releasing factor in chicks. Comp Biochem Physiol, Part A Mol Integr Physiol 2007;147:173–8.

Taylor T, Field T. Scientific Farm Animal Production. 9th Ed. Upper Saddle River, NJ: Prentice Hall; 2008.

Timpl P, Spanagel R, Sillaber I, Kresse A, Reul JM, Stalla GK, et al. Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor. Nat Genet 1998;19:162–6.

Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotrophin and beta-endorphin. Science 1981;213:1394–7.

Vaughan J, Donaldson C, Bittencourt J, Perrin MH, Lewis K, Sutton S. Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. Nature 1995;378:287–92.

Wong M, Licinio J, Pastern K, Gold P. Localization of corticotrophin-releasing hormone (CRH) receptor mRNA in adult rat brain by in situ hybridization histochemistry. Endocrinology 1994;135:2275–8.

Zhang R, Nakanishi T, Ohgushi A, Ando R, Yoshimatsu T, Denbow DM, et al. Suppression of food intake induced by corticotropin-releasing factor family in neonatal chicks. Eur J Pharm 2001;427:37–41.

Zhao R, Muehlbauer E, Decuypere E, Grossmann R. Effect of genotype-nutrition on growth and somatotropic gene expression in the chicken. Gen Comp Endocrinol 2004;136:2-11.