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Central calcitonin exerts anoretic effects via the hypothalamus in chicks

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ABSTRACT

Calcitonin (CT) causes satiety in mammals, but the mechanisms that mediate this effect are poorly understood. Additionally, there are no reports on CT-induced satiety within the avian class. Therefore, the purpose of this study was to elucidate some of the central mechanisms regulating CT-induced satiety in a non-mammalian vertebrate, the chick. Broiler-type chicks, at 4 days of age, responded to central CT (0.3, 1.0 and 3.0 nmol) with both reduced food and water intake. The effect on water intake was secondary to that of food. An increased number of c-Fos immunoreactive cells were found in hypothalamic nuclei associated with satiety including the arcuate nucleus, dorsomedial nucleus and ventromedial hypothalamus after central CT injection. Increased jumps, distance traveled and time spent perching on food containers were also observed, and these behaviors are likely not competitive with ingestion. Also, central CT injection was associated with reduced food pecks, but increased pecking efficiency. Blockage of corticotrophin releasing factor receptors did not prevent central CT-induced satiety. Central CT appears to be a regulator of satiety in chicks and this effect is likely mediated via interactions within the hypothalamus.

1. Introduction

Calcitonin (CT), composed of 32 amino acids, is primarily produced in C-cells of the thyroid and its release into circulation is triggered by blood borne calcium in mammals (Muff et al., 1995). CT is expressed in the chick central nervous system (Terrado et al., 1998), is cleared by the chick's osteoclasts (Hall et al., 1994), and hypercalcaemia induces its secretion (Baimbridge and Taylor, 1981). CT has long been studied in chicks (Zola et al., 1969) since its original isolation from the mammalian parathyroid (Copp et al., 1962). CT binds to G-protein-coupled seven-transmembrane receptors (Lin et al., 1991), and at least two have been isolated from the rat (Albrandt et al., 1993; Sexton et al., 1993). Additionally, CT receptors have been located in the monkey hypothalamus, near satiety circuitry (Paxinos et al., 2004). CT itself has been isolated from human hypothalamus (Becker et al., 1979).

Thus, it is not surprising that CT affects appetite. Several reports agree that CT causes a potent decrease in food intake in mammals including rats, monkeys and man (Levine and Morley, 1981; Perlow et al., 1980; Freed et al., 1979; Morley et al., 1982a; Wager-Srdar et al., 1986a,b). It is likely that CT is a natural regulator of appetite since its concentration increases following ingestion of a meal (Peng and Gardner, 1980). Levine and Morley (1981) explored some central mechanisms related to CT associated satiety, and found it may be related to altered neuronal calcium flux, and was not likely due to malaise or increased behaviors that are competitive with food intake. Additionally, CT may suppress feeding by antagonism of a naturally occurring orexigenic system such as norepinephrine (Morley et al., 1982b) or insulin (Levine and Morley, 1981) in rats. CT acts as a neuromodulator (Stefăneanu, 1986). However, the hypothalamic mechanisms that mediate CT induced satiety are poorly understood, and CT anorexia has only been studied in mammalian vertebrates.

Therefore, the purpose of the study reported here was to elucidate the hypothalamic mechanisms associated with CT-induced satiety using a member of the closest vertebrae out group to mammals, the chick. We measured food and water intake and counted the number of hypothalamic c-Fos immunoreactive cells after central injection of CT. A comprehensive behavioral analysis was conducted and we attempted to block central CT-induced satiety by inhibiting the hypothalamic–pituitary–adrenal (HPA) axis. The findings presented here provide new insight on the central mechanisms that mediate CT induced satiety.

2. Methods

2.1. Animals

Day of hatch unsexed (male and female) broiler Cobb-500 chicks was obtained from a commercial hatchery. 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 ME/kg) and tap water. All trials were conducted using chicks that were 4 days post hatch. 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 Institutional Animal Care and Use committee. Experiments were

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conducted sequentially as described over a course of 7 weeks. In each experiment chicks were from different hatches.

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 a free-hand injection to be performed. 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 5 s to reduce backflow. Chicks were assigned to treatments at random. Chicken CT (3371.9 molecular weight; American Peptide, Sunnyvale, CA, USA) was dissolved in avian artificial cerebrospinal fluid (Anderson and Heisey, 1972) as a vehicle for a total injection volume of 5 µL with 0.06% 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 through the presence of testes or ovary.

2.3. Experiment 1: effects on food and water intake

Chicks, fasted for 180 min (to intensify the perception of hunger), were randomly assigned to receive either 0 (vehicle only), 0.3, 1.0 or 3.0 nmol CT 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 (measurement accuracy = 0.01 g) every 30 min for 180 min post injection. Water weight (g) was converted to volume (ml; 1 g = 1 ml).

2.4. Experiment 2: effect on water intake in food-restricted chicks

The experimental procedures were identical to those in Experiment 1 except that chicks were not fasted prior to injection, and food was withheld during the observation period. Experiment 2 was conducted 8 days following Experiment 1.

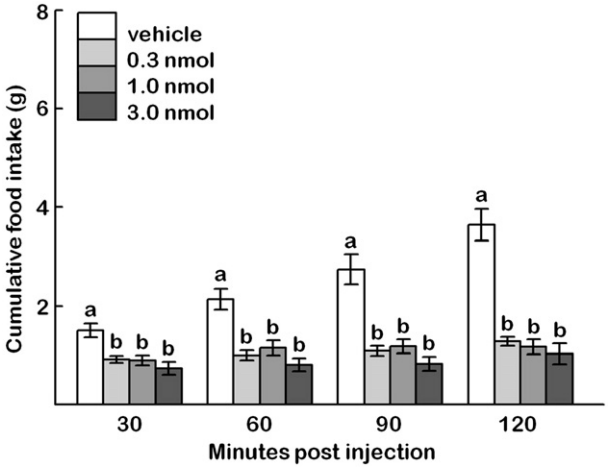

Fig. 1. Cumulative food intake following ICV injection of CT in chicks (Experiment 1). Values are means ± standard error; bars with different superscripts are different from each other within a time point ($P<0.05$). Treatment effects were detected at all time points. Six to 8 chicks per treatment were available for the analysis.

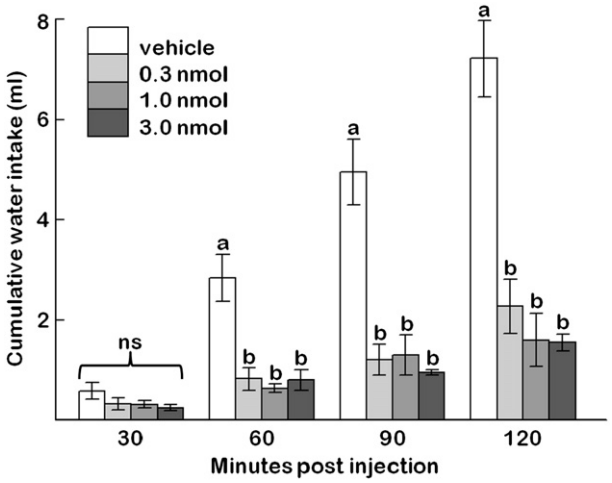

Fig. 2. Cumulative water intake following ICV injection of CT in chicks (Experiment 1). Values are means ± standard error; bars with different superscripts are different from each other within a time point ($P<0.05$). ns, not significant. Six to 8 chicks per treatment were available for the analysis.

2.5. Experiment 3: c-Fos immunoreactive cell counts

Chicks, fasted for 180 min, were randomly assigned to receive either vehicle or 0.3 nmol CT via ICV injection and given *ad libitum* access to both food and water post injection. Thirty minutes after ICV 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 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), superchiasmatic nucleus (SCh), and the ventromedial hypothalamus (VMH). Sections were incubated with anti-Fos polyclonal antibody (1:600, v/v; Sigma, St. Louis, MO, USA; the immunogen corresponds to the N-terminal region of human c-Fos proto-oncogen [p55], amino acids 3–16, and chicken c-Fos has significant homology with this sequence) for 72 h at 4 °C and then with an alkaline phosphatase-conjugated secondary monoclonal antibody (1:600 v/v; Sigma) at room temperature for 3 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 respective nucleus using light microscopy by the first author (J.L.L.) blind to treatment, according to coordinates based on Puelles et al. (2007). The AH, DMN, LH, PaMC, PaMC, SCh, and VMH were collected from within ±0.2 mm of interaural 2.08 mm, and ARC within ±0.2 mm of interaural 1.36 mm. Two sections were counted and averaged to arrive at the value for each chick.

2.6. Experiment 4: behavioral effects

From the day of hatch chicks were kept in individual cages with auditory but not visual contact with each other, and were randomly assigned to receive either vehicle or 0.3 nmol CT by ICV injection. Following 180 min of fasting, injections were made and chicks were immediately placed in a 290 × 290 mm acrylic recording arena with food and water containers (filled to half capacity) in diagonal corners. Individual chicks were simultaneously and automatically recorded from

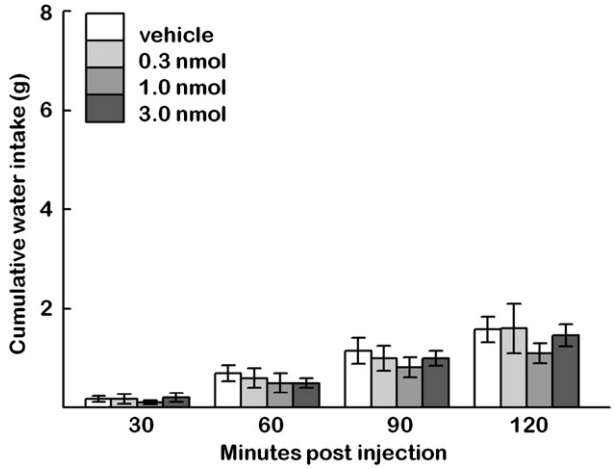

Fig. 3. Cumulative water intake following ICV injection of CT in food restricted chicks (Experiment 2). Values are means ± standard error; bars with different superscripts are different from each other within a time point ($P<0.05$). Treatment effects were detected at all time points. Seven to 10 chicks per treatment were available for the analysis.

three 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). Locomotion (m traveled), the amount of time spent standing, sitting, or in deep rest, and the number of jumps, food and exploratory pecks, defecations and escape attempts were quantified. Food pecks were defined as pecks within the food container, whereas any other pecks were counted as exploratory. Deep rest was defined as the eyes closed for greater than 3 s, starting 3 s after eye closure. Pecking efficiency was calculated by dividing food consumed by number of food pecks for each chick.

2.7. Experiment 5: blockade of corticotrophin releasing factor (CRF) receptors and CT-induced anorexia

The experimental procedures were identical to those in Experiment 1 except that chicks were randomly assigned to receive either vehicle, 0.3 nmol CT, 6.0 nmol astressin or 0.3 nmol CT + 6.0 nmol astressin via ICV injection.

2.8. Statistical analysis

Data from Experiments 1, 2 and 5 were analyzed using analysis of variance (ANOVA) at each time point using the GLM procedure of SAS. The model included dose, sex and the interaction of dose with sex. Sex was not significant in any experiment, thus it was removed from the model. When significant treatment effects were found, Tukey's method of multiple comparisons was used to separate the means at each time period.

Data from Experiment 3 were analyzed comparing vehicle- to CT-treated chicks by ANOVA using the GLM procedure of SAS. Behavior data from Experiment 4 were non-parametric and thus were analyzed by the by the Wilcoxon rank-sum test using the NPAR1WAY procedure of SAS. Pecking efficiency data from Experiment 4 were analyzed using ANOVA by the GLM procedure of SAS. In all experiments statistical significance was set at $P<0.05$.

3. Results

3.1. Experiment 1: effects on food and water intake

Chicks responded to central CT with a potent statistically different decrease in food intake that was common of dose (Fig. 1). The effect of central CT on food intake was significant at 30 min and remained significant throughout the end of the observation period. As time progressed, treatment divergence of the vehicle- and CT-treated chicks increased; compensatory food intake was not observed. Although food intake was significantly decreased in all groups of chicks that received CT, they continued to consume food between observation times. Food intake was not affected by sex or sex by CT dose interaction. Water intake was also affected (Fig. 2). Chicks treated with central CT had reduced water intake by 60 min and thereafter, and this effect was not dependant on dose. Like food intake, divergence of the vehicle- and CT-treated chicks increased as time progressed, and there was not compensatory water intake. Water

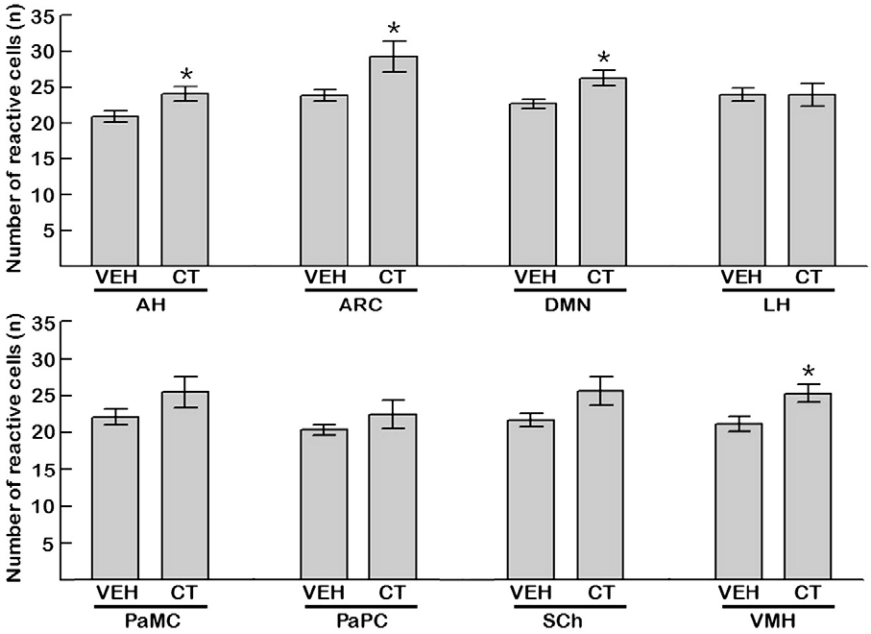

Fig. 4. Effect of ICV injection of CT on the number of c-Fos immunoreactive cells in the anterior hypothalamus (AH), arcuate nucleus (ARC), dorsomedial nucleus (DMN), lateral hypothalamus (LH), magnocellular division of the paraventricular nucleus (PaMC), parvicelluar division of the paraventricular nucleus (PaPC), superchiasmatic nucleus (SCh), and the ventromedial hypothalamus (VMH) in chicks (Experiment 3). (*) denotes different from control ($P<0.05$). Values are means ± standard error. Five chicks per treatment were available for the analysis.

intake was not affected by sex or sex by CT dose interaction. Six to 8 chicks per treatment were available for the analysis.

3.2. Experiment 2: effect on water intake in food-restricted chicks

When chicks were food restricted during the observation period, central CT did not affect water intake (Fig. 3). Seven to 10 chicks per treatment were available for the analysis.

3.3. Experiment 3: number of c-Fos immunoreactive cells

Central CT affected the number of hypothalamic c-Fos immunoreactive cells (Fig. 4). Chicks that received central CT had statistically increased activity in the AH, ARC, DMN and VMH; the magnitude of activation was 15%, 23%, 16% and 19% respectively over vehicle-treated chicks. There was no effect detected in LH, PaMC, PaPC or the SCh. Five chicks per treatment were available for the analysis.

3.4. Experiment 4: behavioral effects

Chicks that received central CT responded with statistically different count-type behaviors (Table 1). Pecks at food were statistically reduced by 900 s and throughout the remainder of observation. Additionally, jumps were increased at 900 and 1200 s and total distance moved was increased at 900 s. Other behaviors including exploratory pecking, escape attempts and defecations were not affected by central CT. A timed-type behavior was also affected by central CT injection (Table 2). At 900 s CT-treated chicks perched longer than vehicle-treated chicks. Other timed behaviors including standing, sitting, and deep rest time were not affected by central CT. During the behavior observation CT-treated chicks consumed statistically less food than vehicle-treated chicks (2.06 ± 0.2 g vs. 3.3 ± 0.4 g respectively), however pecking efficiency was increased by CT treatment (0.004 ± 0.0004 g/peck vs. 0.003 ± 0.0002 g/peck, respectively). Nine vehicle- and 6 CT-treated chicks were available for the analysis.

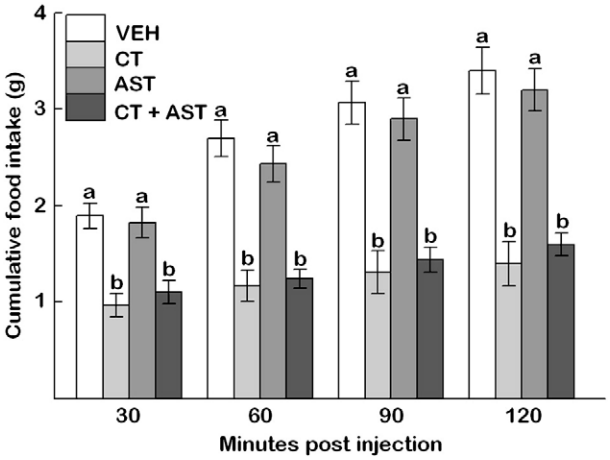

Fig. 5. Cumulative food intake following ICV injection of either vehicle (VEH), calcitonin (CT), astressin (AST), or CT + AST (Experiment 5). Values are means ± standard error. Seven to 10 chicks per treatment were available for the analysis.

3.5. Experiment 5: blockade of CRF receptors and NPK-induced anorexia

In Experiment 5, chicks treated with CT had a similar magnitude of food intake reduction as observed in Experiment 1 (Fig. 5). Astressin, which blocks CRF receptors, did not statistically affect food intake. However, when CT and astressin were given together food intake was statistically reduced, a reduction with similar magnitude to the group of chicks that received CT alone. Seven to 10 chicks per treatment were available for the analysis.

Table 1
Count-type behaviors following ICV injection of CT in chicks (Experiment 4).

Behavior	Treatment	Time post injection (s)					
		300	600	900	1200	1500	1800
Feeding pecks (n)	Vehicle	186 ± 54	513 ± 126	819 ± 155	1011 ± 204	1202 ± 258	1288 ± 282
	Calcitonin	235 ± 54	360 ± 100	442 ± 108*	499 ± 121*	515 ± 128*	531 ± 136*
Exploratory pecks (n)	Vehicle	7 ± 5.2	7.4 ± 5.1	10.2 ± 4.9	16.6 ± 6	19.2 ± 6.8	21 ± 7.7
	Calcitonin	2.5 ± 0.8	6.6 ± 2.7	8.3 ± 3.8	14 ± 6.5	17 ± 7.1	19.5 ± 7.3
Jumps (n)	Vehicle	0.7 ± 0.3	1.4 ± 0.6	1.6 ± 0.7	2.2 ± 0.7	3.3 ± 1.0	5.3 ± 1.3
	Calcitonin	2.5 ± 2.1	6.1 ± 4.4	7.8 ± 4.1*	10.6 ± 4.5*	13.5 ± 5.4	14.5 ± 6.1
Distance moved (m)	Vehicle	1.5 ± 0.3	2.0 ± 0.6	2.4 ± 0.7	4.2 ± 1.6	5.9 ± 2.4	7.2 ± 2.6
	Calcitonin	1.0 ± 0.5	3.2 ± 1.3	4.6 ± 1.3*	6.3 ± 1.5	8.3 ± 1.6	10.1 ± 2.0
Escape attempts (n)	Vehicle	0.6 ± 0.3	1.0 ± 0.5	1.1 ± 0.5	1.6 ± 0.5	2.4 ± 0.7	4.1 ± 1.0
	Calcitonin	2.3 ± 1.9	5.8 ± 4.3	6.6 ± 4.2	9.1 ± 4.2	11.8 ± 4.8	13 ± 5.4
Defecation (n)	Vehicle	0.2 ± 0.1	0.4 ± 0.2	0.4 ± 0.2	0.6 ± 0.3	0.6 ± 0.2	0.7 ± 0.2
	Calcitonin	0	0.3 ± 0.2	0.3 ± 0.2	0.8 ± 0.3	0.8 ± 0.3	0.6 ± 0.2

Values are means ± standard error. Significance from vehicle is indicated by (*) which implies $P<0.05$. Nine vehicle- and 6 calcitonin-treated chicks were available for the analysis.

Table 2
Timed-type behaviors following ICV injection of CT in chicks (Experiment 4).

Behavior	Treatment	Time post injection (s)					
		300	600	900	1200	1500	1800
Stand time (s)	Vehicle	298 ± 13	597 ± 18	897 ± 19	1194 ± 28	1490 ± 34	1789 ± 41
	Calcitonin	298 ± 9.5	595 ± 24	893 ± 27	1190 ± 21	1489 ± 28	1787 ± 48
Sit time (s)	Vehicle	0	0	0	0	0	0.3 ± 0.2
	Calcitonin	0	0	0	0	0	0
Deep rest (s)	Vehicle	0	0	0	0	0	0.4 ± 0.4
	Calcitonin	0	0	0	0.3 ± 0.3	0.5 ± 0.5	1 ± 1
Perch time (s)	Vehicle	1 ± 0.6	1.4 ± 1	2.5 ± 1	4.1 ± 1.3	5.5 ± 1.6	6.8 ± 2.13
	Calcitonin	1.4 ± 1	3.1 ± 1.6	5.8 ± 1.8*	7.6 ± 1.9	8.8 ± 2.4	9.3 ± 2.67

Values are means ± standard error. Significance from vehicle is indicated by (*) which implies $P<0.05$. Nine vehicle- and 6 calcitonin-treated chicks were available for the analysis.

4. Discussion

The results presented here demonstrate that central CT causes anorexigenic effects in chicks, as it does in mammals, and thus these effects are likely conserved across multiple species. The CT we injected (CASLSTCVLGKLSQELHKLQTYPRTDVGAGTP) is 100% identical to chicken CT (accession EU367492.1).

In Experiment 1, chicks responded to central CT with reduced food intake, and this effect was significant at even the lowest dose tested. Thus, CT may be a more potent satiety signal than other peptides tested in chicks that require higher doses including NPVF, NPFF, and xenin (Cline et al., 2008, 2007a,b). Some neurotransmitters only affect short term satiety such as NPFF or NPVF in chicks, unlike CT which may exert its effects long term. With others such as insulin (Honda et al., 2007) and xenin (Cline et al., 2007b) there is a brief lag time before anorexigenic effects are detected, which is not the case for CT. In rats, ICV CT administered at a dose slightly under half of our lowest dose tested completely abolished food intake for 8 h (Levine and Morley, 1981). The magnitude of food intake suppression in the present study at 60 min after injection is similar to that reported by Freed et al. (1979) in rats 24 h after injection (the first observation time in that study). Additionally, a single injection of CT caused reduced food intake in rats for up to 5 days, and 3 days in monkeys (Perlow et al., 1980). Thus, central CT may also exert long term effects in chicks.

Water intake was not measured in the ICV rodent studies, but subcutaneous CT caused increased water intake and pronounced dieresis (Perlow et al., 1980). Our results show water intake was reduced in CT-treated chicks under *ad libitum* feeding. Therefore, thirst is differentially affected by CT in rodents and chicks. We speculated that the effect on water intake may be secondary to that on food intake, and this hypothesis was supported by the results of Experiment 2. Additionally, since chicks continued to drink in Experiment 2, the anorexigenic effect observed in Experiment 1 is not likely due to malaise.

The ARC, DMN and VMH are associated with satiety perception (Bernardis, 1975; Kalra et al., 1999; Brobeck, 1946) and had an increased number of c-Fos immunoreactive cells in Experiment 3 after CT injection. This may be interpreted as the hypothalamus mediates the anorexigenic effects that were observed in Experiment 1. deBeaurepaire and Freed (1987) reported that some rats responded to infusion of CT in the AH with decreased eating, which is consistent with the effect we observed in chicks. Additionally, CT infusion into the superior VMH caused a 79% anorexia, consistent with the increased activation in the chick VMH after CT treatment. However, infusion of CT into the paraventricular nucleus caused the most profound suppression of rat food intake (deBeaurepaire and Freed, 1987), which is inconsistent with our results. This implies that hypothalamic signaling after central CT differs between rats and chicks. Since the LH was not affected in the present study, the effects of CT may be mediated via effects on satiety perception and not suppression of hunger (Brobeck, 1946; Anand and Brobeck, 1951).

That food intake was reduced in Experiment 4 supports the anorexigenic effects measured in Experiments 1 and 3. The design of Experiment 4 was intended to determine if CT caused behaviors that may be competitive with food intake. The increased jumping and distance moved that were observed may be considered competitive with food intake since food was available in a single container; however, perch time was also increased and in all cases perching occurred on the food not water container (which were in diagonal corners). Chicks pecked at food while perched, thus perching may be viewed as a facilitator of feeding. Since both competitive and facilitator behaviors were increased by central CT injection, the effect on food intake may be primary while other behaviors are secondary. This, taken together with stimulation of satiety related nuclei in Experiment 3, may be interpreted as CT's central behavioral effect is primary on appetite. In mice, CT did not cause disruption of behavior, although this was not an effect statistically tested (Morley et al., 1982c). Additionally, CT did not affect rat chewing behavior (Levine and Morley, 1981), unlike in the present study where feeding efficiency was increased. Therefore, CT affects on behaviors unrelated to ingestion may differ between rodents and chicks.

Increased jumping and distance moved that was observed in Experiment 4 may be interpreted as central CT anorexigenic effects are mediated through activity of the HPA axis. This hypothesis was tested in Experiment 5 through blockade of CRF receptors. Since treatment with astressin did not affect CT-induced reduced food intake, if CT does affect the HPA in chicks it is not of enough magnitude to affect appetite.

In sum, we have found that ICV injection of CT causes a potent reduction of food intake in chicks. While water intake is also reduced (an effect opposite that of rats), it is secondary to food intake. It is likely that the hypothalamus primarily mediates these effects since nuclei associated with satiety perception including the ARC, DMN and VMH were stimulated by ICV CT. The affect on appetite caused by CT is likely behavior specific, and secondary behaviors such as perching, jumping and distance travel may replace ingestion. Although CT causes a reduction in appetite, it causes chicks to consume more food with each peck. Lastly, we demonstrated that CT-induced satiety is not mediated via the HPA axis. Taken together, our findings suggest that CT is a potent regulator of the chick's appetite, with some similarities and differences between mammalian systems.

References

Albrandt K, Mull E, Brady EM, Herich J, Moore CX, Beaumont K. Molecular cloning of two receptors from rat brain with high affinity for salmon calcitonin. FEBS Lett 1993;325:225–32.

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.

Baimbridge KG, Taylor TG. The role of calcitonin in controlling hypercalcaemia in the domestic fowl (*Gallus domesticus*). Comp Biochem Physiol Part A Mol Integr Physiol 1981;68:647–51.

Becker KL, Snider RH, Morre CF. Calcitonin in extrathyroidal tissues of man. Acta Endocrinol 1979;92:746–51.

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;26:541–59.

Cline MA, Nandar W, Rogers JO. Central neuropeptide FF reduces feed consumption and affects hypothalamic chemistry in chicks. Neuropeptides 2007a;41:433–9.

Cline MA, Nandar W, Rogers JO. Xenin reduces feed intake by activating the ventromedial hypothalamus and influences gastrointestinal transit rate in chicks. Behav Brain Res 2007b;179:28–32.

Cline MA, Bowden CB, Calchary WA, Layne JE. Short-term anorexigenic effects of central neuropeptide VF are associated with hypothalamic changes in chicks. J Neuroendocrinol 2008;20:971–7.

Copp DH, Cameron EC, Cheney BA, Davidson AG, Henze KG. Evidence for calcitonin—a new hormone from the parathyroid that lowers blood calcium. Endocrinology 1962;70:638–49.

Davis J, Masuoka D, Gerbandt L, Cherkin A. Autoradiographic distribution of l-proline in chicks after intracerebroventricula injection. Physiol Behav 1979;22:693–5.

deBeaurepaire R, Freed WJ. Anatomical mapping of the rat hypothalamus for calcitonin-induced anorexia. Pharmacol Biochem Behav 1987;27:177–82.

Freed WJ, Perlow MJ, Wyatt RD. Calcitonin: inhibitory effect on eating in rats. Science 1979;206:850–2.

Hall MR, Kief NL, Gilman VR, Gay CV. Surface binding and clearance of calcitonin by avian osteoclasts. Comp Biochem Physiol Part A Mol Integr Physiol 1994;108:59–63.

Honda K, Kamisoyama H, Saneyasu T, Sugahara K, Hasegawa S. Central administration of insulin suppresses food intake in chicks. Neurosci Lett 2007;423:153–7.

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.

Levine AS, Morley JE. Peptidergic control of insulin induced feeding. Peptides 1981;2:261–4.

Lin HY, Harris TL, Flannery MS, Aruffo A, Kaji EH, Gorn A, et al. Goldring SR. Expression cloning of an adenylate cyclase-coupled calcitonin receptor. Science 1991;254:1022–4.

Morley JE, Levine AS, Brown DM, Haandwerger BS. The effect of calcitonin on food intake in diabetic mice. Peptides 1982a;3:17–20.

Morley JE, Levine AS, Murray SS, Kneip J. Peptidergic regulation of norepinephrine induced feeding. Pharmacol Biochem Behav 1982b;16:225–8.

Morley JE, Levine AS, Murray SS, Kneip J, Grace M. Peptidergic regulation of stress-induced eating. Am J Physiol 1982c;243:R159-163.

Muff R, Born W, Fischer JA. Calcitonin, calcitonin gene-related peptide, adrenomedullin and amylin: homologous peptides, separate receptors and overlapping biological actions. Eur J Endocrinol 1995;133:17–20.
Paxinos G, Chai SY, Christopoulos G, Huang XF, Toga AW, Wang HQ, et al. In vitro autoradiographic localization of calcitonin and amylin binding sites in monkey brain. J Chem Neuroanat 2004;27:217–36.
Peng TC, Gardner SC. Sex differences in serum calcitonin level in rats as related to feeding, fasting and age. Endocrinology 1980;107:289–93.
Perlow MJ, Freed WJ, Carman JS, Wyatt RJ. Calcitonin reduces feeding in man, monkeys and rats. Pharmacol Biochem Behav 1980;12:609–12.
Puelles L, Martinez-de-la-Torre M, Paxinos G, Watson C, Martinez S. The chick brain in stereotaxic coordinates: an atlas featuring neuromeric subdivisions and mammalian homologies. 1st ed. Burlington: Academic Press; 2007.
Sexton PM, Houssami S, Hilton JM, O'Keeffe LM, Center RJ, Gillespie MT, et al. Identification of brain isoforms of the rat calcitonin receptor. Mol Endocrinol 1993;7:815–21.
Stefăneanu L. Calcitonin as a neuromodulator. Endocrinologie 1986;24:49–50.
Terrado J, Gerrikagoitia I, Raldúa D, Sorribas V, Martínez-Millán L, Sarasa M. The two mature transcripts of the chick calcitonin gene are expressed within the central nervous system during embryogenesis. Mech Dev 1998;77:81–4.
Wager-Srdar SA, Morley JE, Levine AS. The effect of cholecystokinin, bombesin and calcitonin on food intake in virgin, lactating and postweaning female rats. Peptides 1986a;7:729–34.
Wager-Srdar SA, Morley JE, Levine AS. Calcitonin as a neuromodulator. Endocrinologie 1986b;24:49–50.
Zola H, Phillips AW, Mackinnon SJ, Stewart GA. Some properties of chick ultimobranchial calcitonin. Comp Biochem Physiol 1969;30:783–4.