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REGULAR ARTICLE

Calcitonin is expressed in the chicken pituitary gland: influence of gonadal steroids and sexual maturation

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Abstract Calcitonin (CT) is primarily produced by the thyroid C cells in mammals or by the ultimobranchial gland in chickens. CT is also expressed by the pituitary gland in rats in which it functions as a paracrine factor causing decreased lactotroph proliferation and prolactin (PRL) secretion. Gonadal steroids influence CT expression in the rat pituitary gland. However, the expression of the CT gene in the pituitary gland of chickens or of any other avian species has not previously been reported. We have tested the hypotheses that CT is expressed in the chicken pituitary gland, and that its expression is influenced by sexual maturation or in response to ovarian steroid administration. We have detected robust expression of CT cDNA in the chicken pituitary gland by reverse transcription/polymerase chain reaction (PCR). The sequence of the pituitary-derived CT cDNA is identical to that of the ultimobranchial gland. CT-immunoreactive (ir) cells have been observed throughout the anterior pituitary gland by confocal microscopy. Many of the PRL-ir cells show co-localization with CT-ir cells. Quantitative real-time PCR analysis has revealed an inverse relationship between the quantities of PRL mRNA and CT mRNA in the pituitary gland: sexually mature hens contain lower amounts of CT mRNA but larger quantities of PRL mRNA compared with sexually immature chickens. Estradiol and/or progesterone treatment of sexually immature chickens leads to a significant decrease in the quantity of pituitary CT mRNA relative to that in the vehicle-treated chickens. We conclude that pituitary CT plays an important paracrine/autocrine role in the control of lactotroph function and PRL secretion in the chicken.

Keywords Prolactin · Lactotrophs · Progesterone · Estradiol · Ovary · Chicken (Leghorn)

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Introduction

Calcitonin (CT), a 32-amino-acid peptide hormone, is derived from procalcitonin, which is secreted by the C cells of the thyroid gland in mammalian species or from the ultimobranchial gland of avian species (Copp and Cameron 1961; Pearse 1966; Copp et al. 1967). However, the presence of CT has been documented in several other organs in rats, humans, frogs, rainbow trout, and fugu (Deftos et al. 1980; Clark et al. 2002; Hidaka and Suzuki 2004). For instance, CT expression has been identified in the thymus, jejunum, lung, urinary bladder (Russwurm et al. 2001), prostate (Sjoberg et al. 1980), brain, pituitary gland (Deftos et al. 1978; Flynn et al. 1981), mammary gland (Bucht et al. 1986; Tverberg et al. 2000), endometrium (Ding et al. 1994), and testis (Hidaka and Suzuki 2004) in mammalian species. In avian species, a salmon-CT-like molecule has been detected in the extracts of hypothalamus and mid-brain of pigeons (Galan Galan et al. 1981). Although the function of CT expressed in many of these tissues is not well understood, the induction of CT gene expression has been documented in several extrathyroidal tissues in response to sepsis in hamsters (Muller et al. 2001). The physiological functions of pituitary-derived CT has been well documented. Intrapituitary CT acts as a

paracrine factor to decrease lactotroph proliferation and prolactin (PRL) secretion (Shah et al. 1988, 1999).

The expression of the PRL gene in the anterior pituitary gland and the secretion of PRL undergo dramatic changes with both the onset and the termination of reproductive activity in chickens and turkeys (Shimada et al. 1991; Wong et al. 1991; Kansaku et al. 1994). Intrapituitary CT may play an important role in the modulation of PRL secretion in hens going through changes in reproductive function. Moreover, the avian anterior pituitary gland has distinct cephalic and caudal lobes populated predominantly by lactotrophs and somatotrophs, respectively (Mikami and Yamada 1984). The localization of CT-expressing cells in the pituitary gland should improve our knowledge concerning the possible paracrine role of CT on the secretion of pituitary hormones. The expression of CT, however, has not been described in the pituitary gland of any avian species. Here, we report the expression of CT cDNA in the chicken pituitary gland and the influence of reproductive status or ovarian steroid administration on the quantity of pituitary CT mRNA. Furthermore, we provide evidence that CT is localized to lactotrophs in the chicken pituitary gland.

Materials and methods

Animals

Female Leghorn chickens (16–26 weeks old; Hyline W36 strain) were maintained in cages under a photoperiodic cycle of 16 h light and 8 h dark. The chickens were provided with water and feed ad libitum. All animal procedures were carried out in accordance with the approved protocol of the Institutional Animal Care and Use Committee.

Reverse transcription/polymerase chain reaction

Following decapitation of female chickens by means of a guillotine, their anterior pituitary glands, ultimobranchial glands, and skeletal (pectoral) muscles were isolated and frozen in liquid nitrogen. Total RNA from each pituitary gland was extracted by using an RNeasy kit (Qiagen, Valencia, Calif.) following manufacturer's instructions. Genomic DNA was digested on-column with DNAse-I (Qiagen). The quantity and quality of the total RNA was ascertained by measuring absorbance at 260 and 280 nm with a spectrophotometer. First-strand cDNA was synthesized by reverse-transcription of 1 μg total RNA with d $(T)_{30}$A/G/C A/G/C/T primer and 2 U murine-MuLV reverse transcriptase (New England BioLabs, Beverly, Mass.). Approximately 100 ng single-stranded cDNA was used as a template to amplify a 204-bp product of CT cDNA with the CT1FWD and CT1REV primers (Table 1). A touch-down polymerase chain reaction (PCR) was performed by using the following thermocycle: 94°C for 1 min, 30 cycles of 94°C for 5 s and 72–68°C for 3 min. Annealing and primer extension were performed at 72°C, 70°C, and 68°C during cycles 1–5, 6–10, and 11–30, respectively. The PCR products were subjected to agarose gel electrophoresis and ethidium-bromide staining for visualization. As a negative control, reverse transcription (RT) reactions with 1 μg total RNA from pituitary gland but with no reverse transcriptase (No-RT control) were used as the template in place of single-stranded cDNA. In addition, total RNA extracted from blood cells was subjected to RT-PCR to rule out total RNA from blood as being a source of CT mRNA. The RT-PCR product was gel-purified and sequenced (Davis Sequencing, Davis, Calif.) to confirm the authenticity of the product. With another set of primers (CT2FWD and CT2REV; Table 1), a 417-bp full length CT cDNA was amplified by using chicken pituitary cDNA as a template. The resultant product was sequenced to compare the sequences of pituitary and ultimobranchial CT cDNAs.

Collection of anterior pituitary gland for histology

Sexually immature female chickens (Hyline W36 strain, 16 weeks old) were anesthetized by pentobarbital, and their heads were perfused with physiological saline and 4% paraformaldehyde solution. The anterior pituitary gland was removed from the cranium and stored in 0.1 M phosphate buffer (pH 7.2) at 4°C until further processing. Pituitaries were dehydrated, cleared, and embedded in paraffin. Sagittal pituitary sections were cut at a thickness of 4 μm on a rotary microtome (Microm, Walldorf, Germany), and serial sections were mounted on Superfrost-plus glass slides (VWR, West Chester, Pa.).

Co-localization of CT and PRL in the pituitary gland

Tissue sections of pituitary gland were deparaffinized in Histoclear (National Diagnostics, Atlanta, Ga.) and hydrated in descending concentrations of ethyl alcohol in water. The sections were first blocked with 1% normal goat serum and then incubated at 4°C for about 18 h with a mixture of 1:2,500 mouse anti-chicken synthetic PRL peptide (Berghman et al. 1992) and 1:100 affinity-purified rabbit anti-salmon CT (Peninsula Laboratories, San Carlos, Calif.) antibodies. Following three washes in 0.01 M TRIS-HCl and 0.15 M sodium chloride (pH 7.4; TBS), the sections were incubated at room temperature for 1 h in 1:400 biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, Calif.). After three washes in TBS, the slides were incubated at room temperature for 1 h with 1:250 streptavidin AlexaFlour488

Table 1 Nucleotide sequences of primers used to amplify chicken pituitary calcitonin (*CT*), prolactin (*PRL*), or β-actin complementary deoxyribonucleic acids. GenBank accession numbers and amplicon sizes are also given

Name	Sequence	GenBank accession number	Product length (bp)
CT			
CT1Fwd	5′-AGTCAGACCTGGCTTGGAGTCCATCA-3′	X03012	204
CT1Rev	5′-TGAGACAGTTTGCCCAGCACACAAGT-3′		
CT2Fwd	5′-ATGGTCATGCTGAAGATTTCATCTTTCCT-3′	BMC490287	417
CT2Rev	5′-CTAGTTGTTTCCTAGGGTTTCCCCATAGTT-3′		
PRL			
PRLFwd	5′-AAGAAGCTCCAGATACCATTCTCT-3′	J04614	129
PRLRev	5′-GAGAGTAAATTTCATTTCCAGCAT-3′		
β-Actin			
BAFwd	5′-CTGGCACCTAGCACAATGAA-3′	L08165	123
BARev	5′-CTGCTTGCTGATCCACATCT-3′		

and 1:250 anti-mouse IgG conjugated to AlexaFlour546 (Invitrogen, Carlsbad, Calif.), washed several times in TBS and coverslipped with ProLong Gold antifade (Invitrogen) mounting medium. Green (CT) and red (PRL) fluorescent cells were visualized by exciting the flourophores with respective lasers sequentially on an Olympus FluoView Laser scanning confocal microscope (Olympus, Center Valley, Pa.). For negative controls, pituitary gland tissue sections were incubated with rabbit IgG in place of rabbit anti-salmon CT or mouse anti-chicken PRL IgG.

Quantification of pituitary CT mRNA and PRL mRNA in sexually immature and mature female chickens

The anterior pituitary glands of sexually immature Leghorn chickens (16 weeks old; $n=7$) and sexually mature chickens (26 weeks old; $n=7$) maintained under identical photoperiod were collected as previously described, frozen in liquid nitrogen, and stored at −80 C. Total RNA was extracted from the anterior pituitary as described above. Total RNA (1 μg) was reverse-transcribed with $d(T)_{30}$A/G/C A/G/C/T, 2U M-MLV reverse transcriptase (New England BioLabs) in a 20-μl reaction. Chicken CT mRNA or PRL mRNA and chicken β-actin mRNA were quantified by utilizing 5 μl of the RT reaction (equivalent to 100 ng single-stranded cDNA) as a template in the real-time quantitative PCR. A 204-bp product for chicken CT cDNA was amplified with the CT1FWD and CT1REV primers (Table 1). For quantifying PRL mRNA, a 129-bp partial PRL cDNA corresponding to nucleotides 446-574 (GenBank accession no. J04614) was amplified with the PRLFWD and PRLREV primers (Table 1). Similarly, a 123-bp product of chicken β-actin was amplified with the BAFWD and BAREV primers (Table 1) in separate reactions. The real-time-quantitative PCR consisted of 1× Platinum SYBR Green qPCR Super Mix-UDG (Invitrogen) and 300 nM forward and reverse primers. The reactions were carried out in a DNA Engine Opticon II (MJ Research) under the following thermocycling conditions: 50°C for 2 min, 95°C for 2 min, followed by 35 cycles of 95°C for 15 s, 55°C for 30 s, and 72°C for 30 s. At the end of the amplification, a melting curve analysis was carried out by heating the PCR products from 65°C to 95°C, held for 15 s at increments of 0.2°C, and fluorescence was detected to confirm the presence of a single amplification product. Tissue samples from each animal were run in duplicate to obtain average C_T values for CT mRNA or PRL mRNA and β actin mRNA. As negative controls, RT reactions with 1 μg total RNA with no reverse transcriptase were used as a template in place of single-stranded cDNA in the real-time quantitative PCR. The log-linear threshold values (C_T) during the exponential phase of the PCR for CT or PRL mRNA were subtracted from that of β-actin mRNA. CT mRNA or PRL mRNA quantity was expressed as a proportion of β-actin mRNA quantity following the $2^{-\Delta\Delta C}{}_T$ method for converting log-linear C_T values to linear terms (Livak and Schmittgen 2001). The relative amounts of CT mRNA or PRL mRNA in the anterior pituitary gland were then compared.

Effect of ovarian steroids on quantity of pituitary CT mRNA

Sexually immature female chickens (16 weeks old; $n=6$–7) were injected intramuscularly with peanut oil containing estradiol-17β (0.5 mg/kg body weight; four injections on alternate days; Dunn et al. 2003), progesterone (0.17 mg/kg bodyweight/day for 7 consecutive days; Liu and Bacon 2005), estradiol and progesterone together at the above dosages, or no steroids (negative control). On the seventh day after the first dose of steroids, the chickens were killed as described previously. The oviduct was isolated (infundibulum to shell gland) and weighed to confirm the efficacy of estradiol and/or progesterone treatments. The anterior

pituitary gland was isolated and snap-frozen in liquid nitrogen. Total RNA from each chicken anterior pituitary gland was extracted and subjected to quantitative PCR for the determination of CT mRNA as described above.

Statistical analyses and software used

The relative amount of CT mRNA or PRL mRNA to the amount of β-actin mRNA was first converted from log-linear to linear terms and then compared between sexually immature and mature chickens by Student's *t*-test (SAS; SAS Institute, Cary, N.C.). Correlations between pituitary CT mRNA and PRL mRNA abundance in sexually immature and mature chickens were tested by Pearson correlation coefficients by using the SAS package. Anterior pituitary gland CT mRNA quantity relative to β-actin mRNA quantity among animals treated with vehicle, estradiol, progesterone, and estradiol+progesterone was compared by an analysis of variance by using SAS software after confirmation of homogeneity of variance. A probability level of $P<0.05$ was considered statistically significant. For DNA sequence analysis and for PCR primer design, we used Vector NTI suite 9.1 (Invitrogen).

Results

RT-PCR results

A 204-bp product corresponding to nucleotides 48–251 of a partial CT cDNA (GenBank accession no. X03012) was amplified from the single-stranded cDNA reverse-transcribed from total RNA extracted from anterior pituitary gland and the ultimobranchial gland (Fig. 1). The nucleotide sequences of this 204-bp product and another 417-bp pituitary full-length CT cDNA amplified with the CT2FWD and CT2REV primers (Table 1) revealed significant homology (greater than 99% identities) to CT from chicken ultimobranchial gland (GenBank accession No. X03012). CT cDNA transcripts could not be amplified from total RNA extracted from blood and skeletal muscle (Fig. 1) suggesting that blood cells did not contribute to the amplification of pituitary CT cDNA. The integrity of blood and skeletal muscle RNA or cDNA was confirmed by successfully amplifying a fragment of β-actin cDNA (data not shown). Control samples of reactions without reverse transcriptase did not amplify any product indicating that genomic DNA did not contribute to the amplification of the CT cDNA product (Fig. 1).

Co-localization of CT and PRL in pituitary gland

CT-immunoreactive (ir) cells (Fig. 2a) were scattered throughout the cephalic and caudal lobes of the anterior pituitary gland, whereas PRL-ir cells were found exclusively in the cephalic lobe (Fig. 2b). Although many of the CT-ir cells in the cephalic lobe co-localized with PRL-ir cells (Fig. 2c), several CT-ir cells did not exhibit this co-localization. Both CT and PRL immunofluorescence were restricted to the cytoplasm of the pituitary cells. No staining was observed when rabbit IgG was used in place of anti-CT or anti-PRL IgG during immunohistochemistry (Fig. 2d).

Pituitary CT mRNA and PRL mRNA by quantitative PCR

A significantly higher amount of CT mRNA was observed in sexually immature female chicken pituitary gland compared with mature chickens ($P<0.05$; Fig. 3a). In contrast, the amount of PRL mRNA was significantly higher in sexually mature chickens compared with immature chickens (Fig. 3b). A statistically significant negative correlation of pituitary CT mRNA and PRL mRNA abundance was observed in both sexually immature and mature chickens (Pearson's r=-0.72; $P=0.008$). Melting curve analyses showed the presence of a single PCR product for CT mRNA or PRL mRNA, confirming the specificity of the reaction (data not shown).

Effect of ovarian steroids on amount of pituitary CT mRNA

Oviduct weight significantly increased ($P<0.05$) in chickens treated with estradiol, progesterone, or a combination of estradiol and progesterone, when compared with that of vehicle-treated chickens (data not shown). Estradiol, progesterone, or a combination of estradiol and progesterone treatments resulted in a significant decrease ($P<0.05$) in the amount of CT mRNA in the anterior pituitary gland when

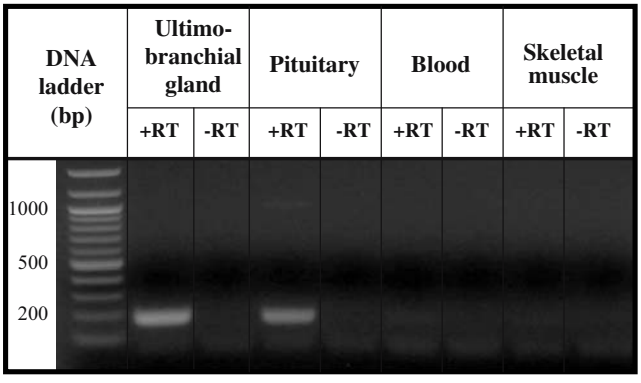

Fig. 1 Reverse transcription/polymerase chain reaction analysis of calcitonin (CT) cDNA expression in chicken ultimobranchial gland, pituitary gland, and blood. Total RNA extracted from the ultimobranchial gland, pituitary gland, and blood was digested by DNAse-I and reverse-transcribed. Approximately 100 ng cDNA (+*RT*) was used as template to amplify a 204-bp CT cDNA product. Contamination controls consisted of RNA from each tissue without reverse transcriptase (−*RT*) or the substitution of water for the template (*bp* base pair)

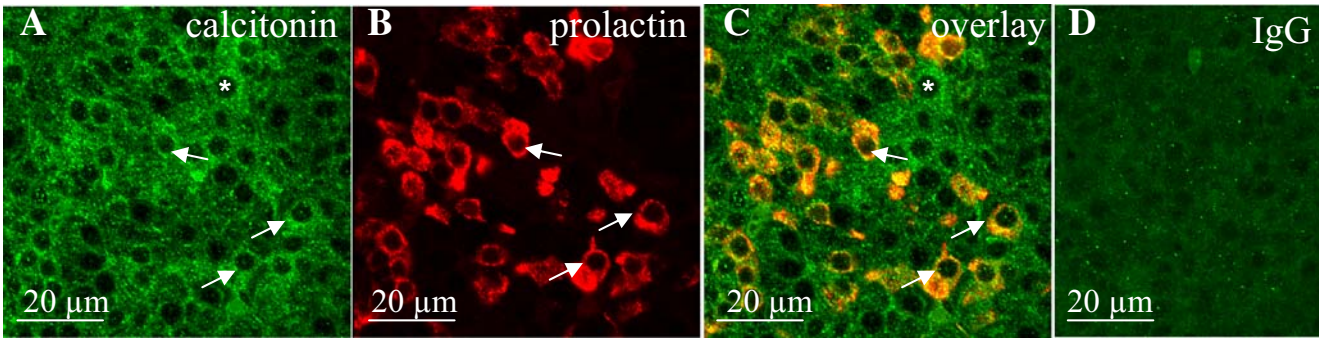

Fig. 2 Photomicrographs of tissue sections of chicken anterior pituitary gland showing cells immunoreactive for calcitonin (CT; **a**, **c**) and prolactin (PRL; **b**, **c**). Pituitary gland tissue sections were immunostained with rabbit anti-CT IgG and mouse anti-PRL IgG. Cells positive for CT (*green*) and PRL (*red*) were visualized by using Alexa488 and Alexa546 flourophores, respectively, and a laser confocal microscope (*arrows* representative pituitary cells that co-localize PRL and CT, *asterisks* nucleus of a CT-immunoreactive cell that does not show co-localization with PRL). **d** Photomicrograph of pituitary tissue section immunostained as above but using rabbit IgG in place of the primary antisera as a negative control

compared with that in vehicle-treated chickens (Fig. 4). Melting curve analyses showed the presence of a single PCR product for CT mRNA or β-actin mRNA confirming the specificity of the reaction (data not shown).

Discussion

This is the first report describing the expression of CT in the pituitary gland of any avian species. We have detected a robust expression of CT cDNA in the pituitary glands of female chickens. The CT cDNA sequence derived from chicken pituitary has been found to be identical to that of a partial CT cDNA sequence obtained from the ultimobranchial gland of the chicken (Lasmoles et al. 1985). In support of our findings, a chicken hypothalamus-pituitary-pineal expressed-sequence-tag library contains a CT cDNA sequence (GenBank accession no. BMC490287) that is almost identical to the full-length 417-bp pituitary-derived CT cDNA. Similar to chickens, the CT cDNA sequence derived from rat pituitary shows greater than 99% homology to the mouse thyroidal CT mRNA sequence (Ren et al. 2001). Collectively, the CT gene expressed in the chicken pituitary gland seems to be homologous to that expressed in the thyroid or ultimobranchial gland in mammalian and avian species, respectively. Two forms of CT were once thought to be produced in the chicken ultimobranchial gland: one resembling salmon CT and another human-CT-like peptide (Perez Cano et al. 1982). CT gene-related peptide (CGRP), a neuropeptide arising from the CT gene by alternative splicing, is expressed in the human brain and pituitary gland (Tschopp et al. 1985) and in the chicken brain (Lanuza et al. 2000). Expression of a CT receptor has been documented in rat pituitary gland (Sheward et al. 1994), whereas radioligand-binding assays with chicken shell gland and kidney membranes suggests the presence of

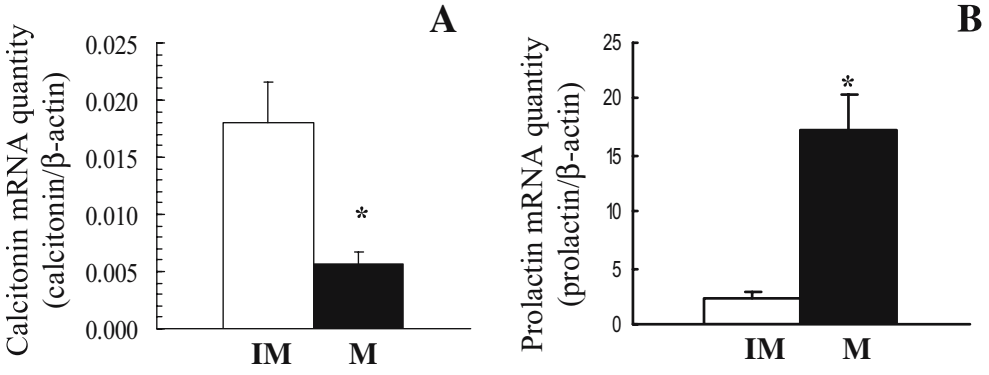

Fig. 3 Amounts of CT mRNA (**a**) or PRL mRNA (**b**) relative to β-actin mRNA in the pituitary gland of sexually immature (*IM*) and mature chickens (*M*; $n=6$). Total RNA from the pituitary gland was treated with DNAse-I and reverse-transcribed. Approximately 100 ng cDNA was used in real-time quantitative PCR with SYBR green as the dye to quantify CT mRNA, PRL mRNA, or β-actin mRNA in separate reactions. Each reaction was run in duplicate and threshold (C_T) values for CT mRNA or PRL mRNA were subtracted from that of β-actin mRNA and converted from log-linear to linear terms. Data represent mean±SE. *Significant difference at $P<0.05$

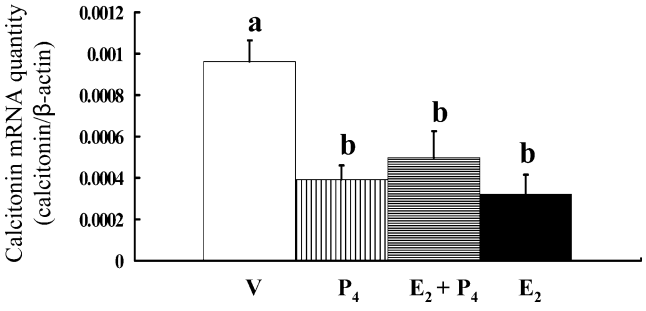

Fig. 4 Effect of estradiol (E_2) and/or progesterone (P_4) on the amount of CT mRNA in the chicken anterior pituitary gland. Sexually immature female chickens were treated with E_2, P_4, E_2+P_4, or vehicle (*V*; n=6-7 per treatment) for 7 days. The chickens were killed by decapitation at the end of treatments. Total RNA extracted from the pituitary gland was subjected to real-time quantitative PCR to determine the amount of CT mRNA, as described in the legend to Fig. 3. Data are means±SE (*a*, *b* significant difference at $P<0.05$)

CT receptors (Yasuoka et al. 1998; Ogawa et al. 2003) in these tissues. However, the expression of a CT receptor in the pituitary gland or other tissues has not been documented.

We have detected CT cDNA in several chicken tissues other than the pituitary gland, such as adipose, diencephalon, kidney, chondrocytes, and ovaries (S.M. Krzysik-Walker and R. Ramachandran, unpublished). Similar to our findings, CT expression has been documented in the thymus, jejunum, lung, urinary bladder (Russwurm et al. 2001), prostate (Sjoberg et al. 1980), brain, pituitary gland (Deftos et al. 1978; Flynn et al. 1981), adipose tissue (Linscheid et al. 2003), mammary gland (Bucht et al. 1986; Tverberg et al. 2000), and endometrium (Ding et al. 1994) in several mammalian species. A clear physiological role of CT in many of these organs has yet to be elucidated. However, both in the pituitary gland and endometrium, CT expression has been associated with regulation of PRL secretion and embryo implantation, respectively (Shah et al. 1988; Kumar et al. 1998). Within the rat pituitary gland, CT has been found to be secreted exclusively by gonadotropes and then acts as a paracrine inhibitor of PRL secretion from lactotrophs (Shah et al. 1993; Ren et al. 2001). Intrapituitary CT has also been shown to decrease lactotroph proliferation (Wang et al. 2003), whereas passive immunoneutralization of CT results in elevated PRL secretion (Shah et al. 1993). Pituitary-derived CT has also been found to decrease promoter activity of the PRL gene in a rat pituitary GGH3 cell line (Ren et al. 2003). More recently, the PRL-inhibitory role of pituitary CT has been further confirmed by the chronic hypoprolactinemia observed in transgenic mice whose pituitary gonadotropes have been engineered to hypersecrete CT (Yuan et al. 2005).

To extend our pituitary CT cDNA expression data to the peptide level, we have determined the localization of CT-ir cells and their possible relationship to PRL-ir cells in the chicken pituitary gland. The avian pituitary gland is organized into distinct cephalic and caudal lobes that predominantly contain lactotrophs and somatotrophs, respectively (Mikami and Yamada 1984). We have found that CT-ir cells are distributed throughout the cephalic and caudal lobes. In the cephalic lobe, many of the CT-ir cells co-localize with PRL-ir cells, suggesting a possible autocrine or intracrine role for CT on lactotroph function. In contrast to these results, CT has been shown to be co-expressed with PRL only in gonadotropes but not in lactotrophs of the rat pituitary gland (Shah et al. 1993). However, such CT-containing gonadotropes are juxtaposed to lactotrophs in the rat pituitary gland (Chronwall et al. 1996). Co-expression of CT and PRL in lactotrophs of the chicken pituitary gland may represent species variation. Conversely, the expression of CT within lactotrophs may be viewed as a more efficient system if CT were to regulate lactotroph function in the same way that it does in rat pituitary gland, rather than being secreted from the gonadotropes. Nevertheless, immunofluorescence for CT has been detected in several other cells, in addition to lactotrophs, in the chicken pituitary gland; the phenotype of these cells is unknown at present. Based on this observation, CT is likely to affect the functions of several other pituitary cell types or hormone secretion(s).

This is the first report to document changes in the expression of the CT gene in the pituitary gland associated with sexual maturity. We have found a 3.6-fold decrease in the amount of CT mRNA in the pituitary gland of 26-week-old sexually mature female chickens compared with that in 16-week-old sexually immature chickens. In contrast, the amount of PRL mRNA is 8.5-fold higher in the pituitary glands of sexually mature chickens compared with that of immature chickens, suggesting an inverse correlation between the expression of the CT and PRL genes. Such an inverse relationship between CT mRNA and PRL mRNA abundance has been reported in the rat pituitary gland (Sun et al. 2002). Changes observed in pituitary CT mRNA abundance in the present study need not necessarily translate into CT expression or secretion. Expression of the PRL gene and PRL secretion are increased in egg-laying female turkeys (Wong et al. 1991). Intrapituitary CT or exogenous salmon CT administration acts as a potent inhibitor of PRL secretion (Shah et al. 1993, 1996). Therefore, a decline in CT mRNA abundance could be interpreted as a factor that allows the upregulation of PRL secretion in the sexually mature chicken.

Sexual maturation in the female chicken is associated with several changes in the hypothalamo-pituitary-ovarian axis, changes that may have affected the expression of the CT and PRL genes in the present study. Among these changes, the secretion of gonadal steroids is expected to be upregulated as a result of ovarian follicular development in sexually mature hens. We have therefore determined the influence of

estradiol and progesterone administration on CT gene expression in 16-week-old sexually immature chicken. Our results confirm that pituitary CT mRNA abundance is significantly decreased following administration of estradiol, progesterone, or a combination of estradiol and progesterone in the chicken. Collectively, higher circulating estradiol and progesterone levels found in sexually mature chickens may be considered as one of the factors that lead to a decrease in the amount of pituitary CT mRNA. Consistent with our results, estrogen treatment in ovariectomized rats has been found to decrease pituitary-CT-like immunoreactivity (Li and Shah 1995). Whereas progesterone treatment in ovariectomized rats does not change the amount of CT mRNA in the pituitary gland, a combination of estradiol and progesterone treatment significantly increases the quantity of CT mRNA (Sun et al. 2002). Such differing effects could either be attributed to species variation or reproductive status.

The expression of CT in the pituitary gland appears to be evolutionarily conserved in several vertebrate species, highlighting its importance in the control of pituitary hormone secretion. Further studies are required to elucidate the role of intrapituitary CT on the secretion of various pituitary hormones in the chicken.

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