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Increased Proliferative Activity and Programmed Cellular Death in the Turkey Hen Pituitary Gland Following Interruption of Incubation Behavior[1]

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ABSTRACT

Incubation behavior or broodiness in turkey hens is characterized by ovarian regression, hyperprolactinemia, and persistent nesting. Nest-deprivation of incubating turkey hens results in disruption of broodiness accompanied by a precipitous decline in plasma prolactin (PRL) concentrations. The objective of the present study is to examine cellular changes in the pituitary gland associated with nest-deprivation for 0, 1, 2, 3, 4, or 7 days. Bromodeoxyuridine (BrdU) was administered prior to kill to study proliferative activity. Pituitary tissue sections were immunostained using turkey growth hormone (GH) antibody, and/or chicken PRL peptide antibody, and BrdU antibody. Plasma PRL concentrations declined significantly following nest-deprivation for 1 or more days. The midsagittal pituitary area immunoreactive (ir) to GH was significantly increased while that of PRL was significantly decreased following nest-deprivation for 2 or more days. Terminal deoxy-UTP nick end labeling and PRL-immunostaining revealed an abundance of apoptotic nuclei in both cephalic and caudal lobes of the anterior pituitary gland, suggestive of programmed cellular death of lactotrophs in the pituitary gland of hens nest-deprived for 2 or more days. Mammosomatotrophs were abundant in hens nest-deprived on Day 0 but were absent in hens nest-deprived for 1 or more days. Proliferating (BrdU-ir) cells were significantly abundant in the pituitary cephalic and caudal lobes following nest-deprivation for 1 or more days but were absent on Day 0 or in laying hens. Dual-labeling studies indicated that most of the BrdU-ir nuclei in the caudal lobe were not colocalized in somatotrophs in hens nest-deprived for 1–4 days but did colocalize with GH following 7 days of nest-deprivation. In conclusion, nest-deprivation of incubating turkey hens results in 1) a precipitous decline in plasma PRL concentration, 2) programmed cell death of lactotrophs, 3) disappearance of mammosomatotrophs, 4) increased proliferative activity of pituitary cells, and 5) recruitment of somatotrophs arising primarily from mitosis of nonsomatotrophic cells.

apoptosis, behavior, growth hormone, pituitary, prolactin

INTRODUCTION

Incubation behavior or broodiness, a parental behavior expressed by egg-laying turkey hens, is associated with hyperprolactinemia and regression of ovaries [1]. The avian pituitary gland is unique among several vertebrate classes in that the pars distalis can be divided into two distinct lobes namely cephalic and caudal lobes. Lactotrophs are distributed in the cephalic lobe; somatotrophs are predominantly found in the caudal lobe in the laying hen pituitary gland [2]. We earlier reported that lactotrophs replace somatotrophs in the pituitary caudal lobe while undergoing massive recruitment and hypertrophy during the onset and maintenance of incubation behavior in turkey hens [2]. Mammosomatotrophs, a bihormonal cell that secretes both prolactin (PRL) and growth hormone (GH), were more abundant in the incubating hen than in the laying hen pituitary gland [3]. Pregnancy and lactation in mammals are also associated with hyperprolactinemia and proliferation of lactotrophs [4]. Increased abundance of PRL-secreting cells with a concomitant decrease in GH-secreting cells has been reported in pregnant and lactating rats [5], and this change is reversed to pregestational state following removal of the suckling stimulus. Interruption of lactation in rats results in programmed cell death of lactotrophs in the pituitary gland [6].

Incubation behavior in turkey hens normally requires tactile stimulation from the brood patch by the nest or eggs [7]. Therefore, nest-deprivation of incubating hens should cause cessation of incubation behavior accompanied by a precipitous decline in plasma PRL concentration [8, 9]. There are no reports on the cellular changes in the turkey pituitary gland associated with such a dramatic decline in plasma PRL levels. The objectives of the present study were to investigate the relative abundance of somatotrophs and lactotrophs as well as the proliferation, differentiation, and programmed cell death of cells in the pituitary gland following nest-deprivation of incubating turkey hens for various lengths of time.

MATERIALS AND METHODS

Animals

Commercial large white turkey hens were reared on deep litter pens. Feed and water were provided ad libitum. A photoperiod of 16L:8D was maintained. Egg production and nest visiting were observed and recorded four times a day. Turkey hens that expressed incubation behavior (cessation of egg laying, persistent nesting, and aggressive defense of the nest) for 13 days were selected at random for interruption of broodiness by nest-deprivation (transfer to a wire cage) for 0, 1, 2, 3, 4, or 7 days. Sample size used was three hens in each treatment group except the 3-day group wherein six birds were used. Incubating hens in the 0-day treatment group were not nest-deprived or cage reared but were taken out of the nest-box, a blood sample was collected, and the bird was killed immediately (time elapsed between removal from nest-box and perfusion was 10–15 min). For the remaining treatments, incubating hens were transferred from the nest-box and reared in cages (one

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hen/cage) and were provided with the same length of photoperiod (16L:8D) as in floor pens. Feed and water were provided at all times. Turkey hens were killed as described previously [10] at the end of the respective treatment days. In order to study proliferative activity in the pituitary gland, all hens except those in the 7-day treatment group were given one i.p. injection of 5-bromo-2′-deoxyuridine (BrdU; Sigma Chemical Co., St. Louis, MO; 50 mg/kg body weight) 14 h prior to kill. For the 7-day treatment group, the birds were injected with BrdU twice, first on Day 3 and again 14 h before kill. Blood samples were collected at the time of transferring incubating hens to cages and also just before kill to compare changes in plasma PRL and GH concentrations. Ovaries and pituitary gland were collected and prepared for further studies as described previously [2].

Pituitaries were dehydrated, cleared, and embedded in paraffin. Sagittal pituitary sections were cut at 4 μm thickness using a Leitz rotary microtome (Leitz, Deerfield, IL) and serial sections were mounted on gelatin-coated glass slides. Plasma PRL and GH concentrations were measured by radioimmunoassay [11, 12].

Prolactin- and/or GH Immunohistochemistry

Midsagittal and parasagittal pituitary sections were immunostained to locate PRL-immunoreactive (ir) or GH-ir cells using an indirect immunoperoxidase method as described previously [2]. Tissue sections were deparaffinized and hydrated. Endogenous peroxidase activity was quenched by treatment with 3% hydrogen peroxide in methanol for 30 min. Nonspecific staining was minimized by incubation with 1% goat or horse serum. Tissues were incubated with either turkey GH antibody (1:15 000) or chicken synthetic PRL peptide antibody (1:60 000) overnight. Following washes in 0.01 M Tris-HCl and 0.15 M sodium chloride (TBS; pH 7.4) buffer, biotinylated goat anti-rabbit IgG or biotinylated horse anti-mouse IgG (Vector Labs, Burlingame, CA) was applied for 1 h. Tissues were then treated with 1:200 avidin peroxidase (Vector Labs). Growth hormone- or PRL-immunostaining was detected using 0.001% 3,3′-diaminobenzidine tetrahydrochloride (DAB) and 0.003% hydrogen peroxide (Sigma). Immunostained pituitary sections were studied using a Zeiss Axioplan microscope (Carl Zeiss, New York, NY), and an image analysis was performed. Microscopic images of immunostained midsagittal pituitary sections were captured using an Optronics video camera and electronically stored. Images of pituitary tissue sections at a low magnification (25×) were outlined to obtain a measurement of total sagittal area using Bioquant software (R&M Biometrics Inc., Nashville, TN). The areas of the pituitary gland that immunostained for GH (GH-ir) or PRL (PRL-ir) were similarly outlined and summated. The resulting immunoarea measurements for GH-ir per bird were ascertained based upon an average computed from four midsagittal pituitary sections and expressed as a proportion (%) of the average total pituitary immunoarea of these sections. For controls, chicken synthetic PRL peptide antibody and turkey GH antibody were preadsorbed with turkey PRL and turkey GH, respectively, and used in the immunostaining [2].

Dual immunofluorescent labeling of GH and PRL was carried out as described previously [3] for locating mammosomatotrophs in the pituitary gland of hens nest-deprived for various lengths of time. The distribution as well as the counts of mammosomatotrophs within the midsagittal and parasagittal pituitary sections were ascertained using an Axioplan microscope (Carl Zeiss).

Bromodeoxyuridine Immunohistochemistry

In order to locate BrdU-incorporated nuclei, a biotin-streptavidin peroxidase complex method was used. Paraffin-embedded pituitary tissue sections were deparaffinized and hydrated using routine procedures. Endogenous peroxidase was quenched by treating tissue sections with 3% hydrogen peroxide in methanol for 30 min. Tissue sections were then treated with 2 N hydrochloric acid for 30 min at 37°C. Following 0.1 M sodium borate treatment for 10 min, the tissues were digested with 0.4% pepsin (Sigma) for 30 min at 37°C. After several washes in TBS, tissues were incubated with 1% horse serum in 1% triton X-100 for 30 min at 37°C followed by incubation with 1:750 mouse BrdU antibody (Sigma) for 2 h at 37°C. After three washes in TBS buffer, a 1:400 solution of biotinylated horse anti-mouse Ig was applied to the tissue sections for 30 min at 37°C. Tissue sections were then treated with a 1:200 solution of streptavidin-peroxidase (Boehringer Mannheim, Indianapolis, IN) for 30 min at 37°C. A brown color reaction product was developed using 0.001% DAB and 0.003% hydrogen peroxide solution. Tissue sections were then washed in deionized water, dehydrated, and mounted using Cytoseal. Proliferating cell nuclei identified by BrdU incorporation were studied in both midsagittal and parasagittal tissue sections. Counts of BrdU-ir nuclei from cephalic and caudal lobes were obtained from four midsagittal pituitary sections from each bird.

In order to ascertain whether BrdU incorporation occurred in GH-containing cells, a dual immunofluorescent labeling was performed as described previously [3]. Tissue sections were pretreated as described in single labeling of BrdU but without hydrogen peroxide treatment while using 1% goat serum instead of horse serum for reducing nonspecific staining. Tissue sections were incubated overnight with a mixture of rabbit antibody to turkey GH (1:5000) and mouse BrdU antibody (1:500). Following washes in TBS buffer, a 1:400 solution of goat anti-rabbit IgG was applied for 1 h. Growth hormone (green) and BrdU (red) were detected using avidin fluorescein isothiocyanate (Vector Labs; 25 μg/ml) and goat anti-mouse IgG conjugated to rhodamine red X (1:100; Jackson ImmunoResearch, West Grove, PA).

Terminal Deoxy-UTP Nick End Labeling Assay

Pituitary tissue sections were subjected to TUNEL assay as described previously [13] with some modifications in order to detect apoptotic nuclei. Midsagittal and parasagittal pituitary sections were first deparaffinized and hydrated using routine procedures. Slides were then washed in TBS buffer and incubated with proteinase K (Boehringer Mannheim; 10 μg/ml 10 mM Tris-HCl, pH 7.4) at room temperature for 20 min. Tissue sections were rinsed in deionized water, TBS, and in terminal transferase (TdT) buffer (30 mM Trizma, 140 mM sodium cacodylate, and 1 mM cobalt chloride, pH 6.8). A nucleotide-TdT mixture was prepared freshly in the following proportion: 300 μl TdT buffer, 3 nmol biotin-dUTP (Boehringer Mannheim), 30 units TdT enzyme (Boehringer Mannheim). About 100 μl of the mixture was applied to each slide under a coverslip and incubated at 37°C for 2 h. The reaction was terminated in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and the slides were rinsed in TBS. After blocking

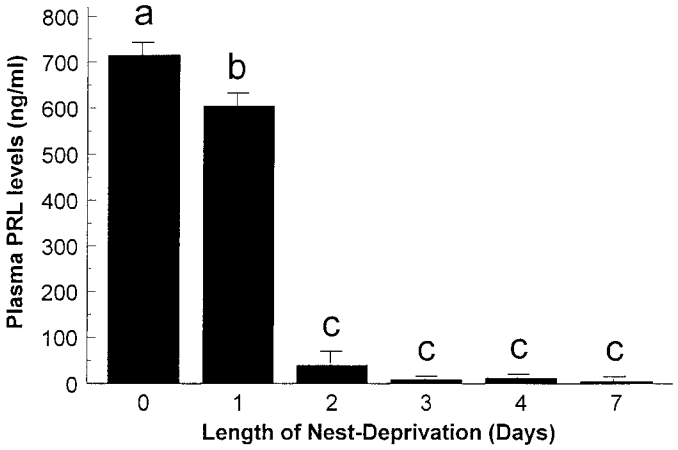

FIG. 1. Plasma prolactin (PRL) concentrations (mean ± SEM) of hens nest-deprived for 0, 1, 2, 3, 4, and 7 days. Sample size used is as follows: three birds for 0-day group, six birds for 3-day group, and three birds each for the rest of the treatment groups. Plasma PRL concentration was estimated in the blood sample collected from every incubating turkey hen at the time of removal from the nest box and the mean hormone level from 21 hens employed in the study is reported in the 0-day group. Significant differences ($P < 0.05$) are denoted by different letters.

nonspecific staining with 5% normal goat serum, tissue sections were incubated with 1:2000 streptavidin-peroxidase (Boehringer Mannheim) at 37°C for 30 min. Following rinsing with 10 mM Tris-HCl (pH 7.4), a color reaction was carried out using 0.001% DAB and 0.003% hydrogen peroxide solution. Slides were then washed in TBS, dehydrated, and mounted using Cytoseal. For negative controls, a TUNEL reaction was run on pituitary tissue sections omitting TdT enzyme. Pituitary sections were treated with DNAse I (Sigma) to create DNA breaks, and a TUNEL procedure was carried out for positive control.

In order to ascertain whether PRL-containing cells undergo apoptosis, a dual labeling procedure was performed to locate both DNA fragmentation and PRL-ir cells in the same pituitary cells. A TUNEL procedure was carried out first, followed by immunostaining using a mouse antibody to chicken synthetic PRL peptide utilizing an indirect immunoperoxidase method described previously [10]. Apoptotic nuclei were labeled with DAB reaction product and PRL granules with Vector-VIP chromogen (Vector Labs).

Statistics

Plasma GH and PRL concentrations, GH immunoarea, PRL immunoarea, and counts of BrdU-ir nuclei were compared across 0, 1, 2, 3, 4, and 7 days of nest-deprivation treatments using GLM procedures of SAS [14]. A probability level of $P < 0.05$ was considered significant.

RESULTS

Plasma Hormone Concentrations

A significant decline in plasma PRL concentration was observed following nest-deprivation for 1, 2, 3, 4, or 7 days compared with 0 days (Fig. 1). There was no significant change in plasma GH concentrations (data not shown). Ovaries of hens in all the treatment groups were completely regressed. Two out of three hens in the 7-day treatment group had a few preovulatory follicles of size less than 10 mm diameter.

Prolactin and GH Immunohistochemistry

Nest-deprivation for 1, 2, 3, 4, and 7 days resulted in a gradual disappearance of PRL-ir cells and emergence of GH-ir cells in the pituitary caudal lobe, a reversal of cellular changes that occurred at the onset of broodiness (Fig. 2, A–D). Recruitment of somatotrophs appeared to progress from the junction of cephalic and caudal lobes and from the ventral caudal lobe toward the central part of the caudal lobe. Somatotrophs that are newly recruited in the ventral part of the caudal lobe show lighter immunostaining when compared with those present in the dorsal part of caudal lobe. A significant increase in the proportion of GH-immunoarea and a significant decrease in PRL-ir was observed in the pituitary gland of incubating turkey hens nest-deprived and cage reared for 2 days or more (Fig. 3). Pread-

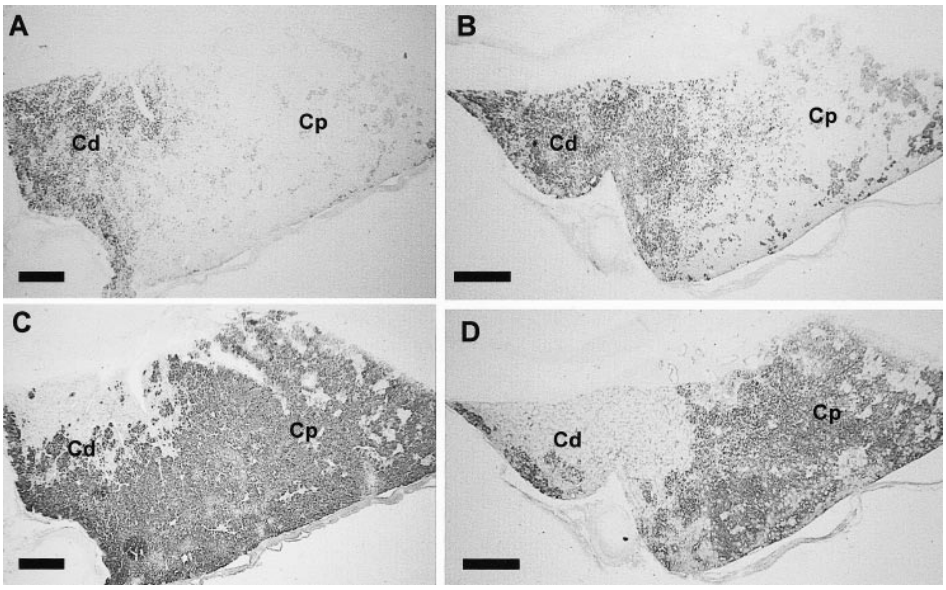

FIG. 2. **A**–**D**) Photomicrographs of midsagittal pituitary sections of incubating hens nest-deprived for 0 days (**A** and **C**) or 3 days (**B** and **D**), showing the distribution of GH-ir cells (**A** and **B**) and PRL-ir cells (**C** and **D**). Cp and Cd refers to cephalic and caudal lobes, respectively. Bars = 100 μm.

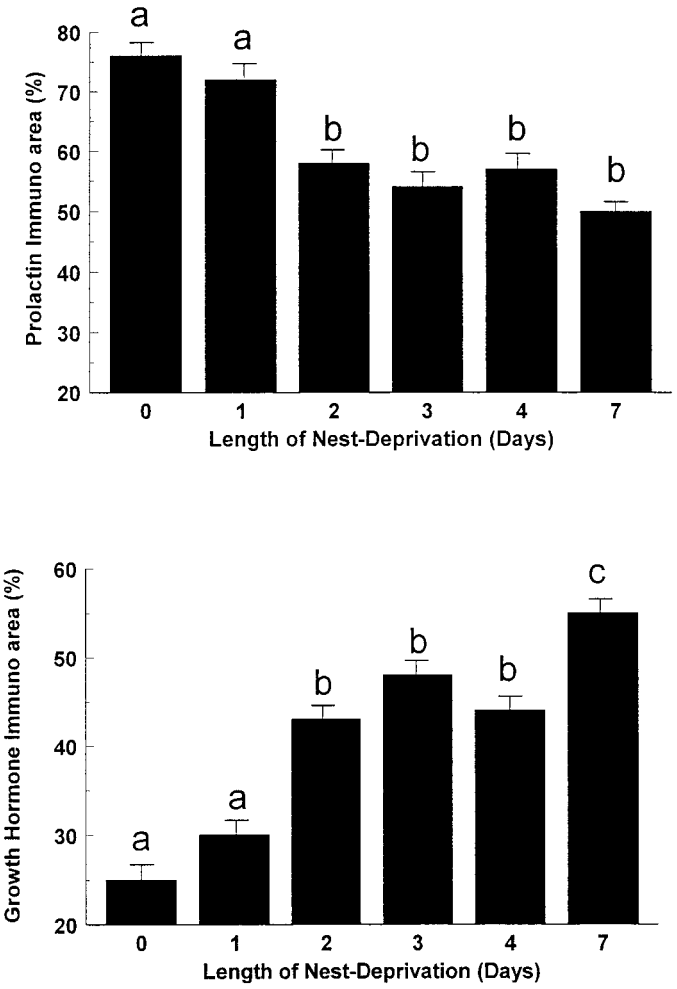

FIG. 3. Prolactin-, or GH-immunoarea in the midsagittal pituitary gland of incubating turkey hens nest-deprived for 0, 1, 2, 3, 4, and 7 days. Midsagittal sections of the anterior pituitary gland were immunostained using anti-turkey GH antibody or anti-chicken synthetic PRL peptide antibody. Prolactin-ir- and GH-ir area as well as total pituitary area were measured by image analysis. A proportion (%) of GH-ir or PRL-ir area to total pituitary area was calculated for every pituitary section (four sections/bird; three or six birds/treatment) and compared among the treatment groups. Bars represent mean GH-ir or PRL-ir area (%) ± SEM. Significant differences ($P < 0.05$) are denoted by different letters.

sorption of anti-chicken synthetic PRL peptide antibody and anti-turkey GH antibody with turkey PRL and turkey GH, respectively, abolished immunostaining [2].

Mammosomatotrophs or GH and PRL colocalized cells were absent in midsagittal and parasagittal pituitary tissue sections of hens nest-deprived for 1 or more days while they were present on Day 0 of nest-deprivation (data not shown). Mammosomatotroph distribution in the pituitary gland tissue sections and their proportion to GH-ir or PRL-ir in the Day 0 treatment group were similar to earlier findings [3].

Bromodeoxyuridine Immunohistochemistry

Proliferating cells, as revealed by BrdU-ir nuclei, were found in cephalic and caudal lobes of pituitary tissue sections of hens nest-deprived for 1 or more days (Fig. 4A) but were absent in the 0-day treatment group as well as in laying hens. At least two loci comprising several BrdU-ir nuclei were found at the junction of cephalic and caudal lobes of pituitary tissue sections of hens nest-deprived for 4 or 7 days. Counts of BrdU-incorporated nuclei revealed an increase in the number of proliferating nuclei in both cephalic and caudal lobes following nest-deprivation for 1 or more days (Fig. 5). Dual labeling procedures revealed BrdU-ir nuclei (red) and GH-immunostaining (green) in separate cells (Fig. 4A) in the caudal lobe of pituitary tissue sections from hens nest-deprived for 1–4 days that were given one dose of BrdU, suggesting that somatotrophs do not undergo mitosis. Instead, there is proliferation of non-somatotrophic cells (Fig. 4A). Administration of two doses of BrdU, on Days 3 and 6 of nest-deprivation, resulted in colocalization of all BrdU-incorporated nuclei in GH-ir cells in the caudal lobe on Day 7 (Fig. 4B). Omission of BrdU antibody in the above staining procedures abolished BrdU immunostaining.

Terminal Deoxy-UTP Nick End Labeling Assay

Nuclear staining by TUNEL was absent in the pituitary gland of hens nest-deprived for 0 days (Fig. 4C). Nest-deprivation for 1, 2, 3, 4, and 7 days resulted in a gradual disappearance of PRL-ir cells and emergence of GH-ir cells in the pituitary caudal lobe, a reversal of cellular changes that occurred at the onset of broodiness. In the 1-day nest-deprived hens, only a few apoptotic nuclei were found in the caudal lobe and at the junction of cephalic and caudal lobes, but none was present in the cephalic lobe. Following nest-deprivation for 2 days or more, numerous apoptotic nuclei were found (Fig. 4D) in the cephalic lobe and caudal lobes. Dual labeling for PRL and apoptotic nuclei revealed lactotrophs with stronger PRL immunostaining in the cytoplasm (black) and no nuclear staining in pituitary sections from the Day 0 treatment group (Fig. 4C). Following nest-deprivation for 4 days, however, there were many apoptotic nuclei in both cephalic and caudal lobes while many of them colocalized in PRL-ir cells (Fig. 4D). Prolactin immunostaining in hens nest-deprived for 2, 3, or 4 days was found to be weaker than in the 0-day treatment group. Omission of TdT enzyme abolished staining in the TUNEL procedure while DNAse I treatment labeled every cell nucleus in the pituitary gland (data not shown).

DISCUSSION

In the present study, we investigated the cellular changes in the pituitary gland associated with a precipitous decline in plasma PRL concentration following nest-deprivation of incubating turkey hens. The decline in plasma PRL concentrations started as early as 1 day of nest deprivation and resulted in an 80-fold decline by 3 days of nest-deprivation. This observation is consistent with earlier studies in turkeys [8, 9]. We had earlier reported that incubation behavior is associated with an increase in the proportion of PRL-ir cells, a decrease in proportion of GH-ir cells [2], and increased abundance of mammosomatotrophs [3]. Interruption of incubation behavior in the present study resulted in a concomitant decline in PRL-immunoarea and an increase in GH-immunoarea. This is largely due to recruitment of somatotrophs that replaced lactotrophs in the caudal lobe, a reversal of cellular changes noticed at the onset, and progression of incubation behavior. Several studies with mammalian pituitaries indicate that there is recruitment of lactotrophs during pregnancy or lactation [4] that is followed by massive regression of redundant lactotrophs upon removal of the stimuli [6]. Progression from nonpregnancy to late lactation in rats is associated with an increase in the

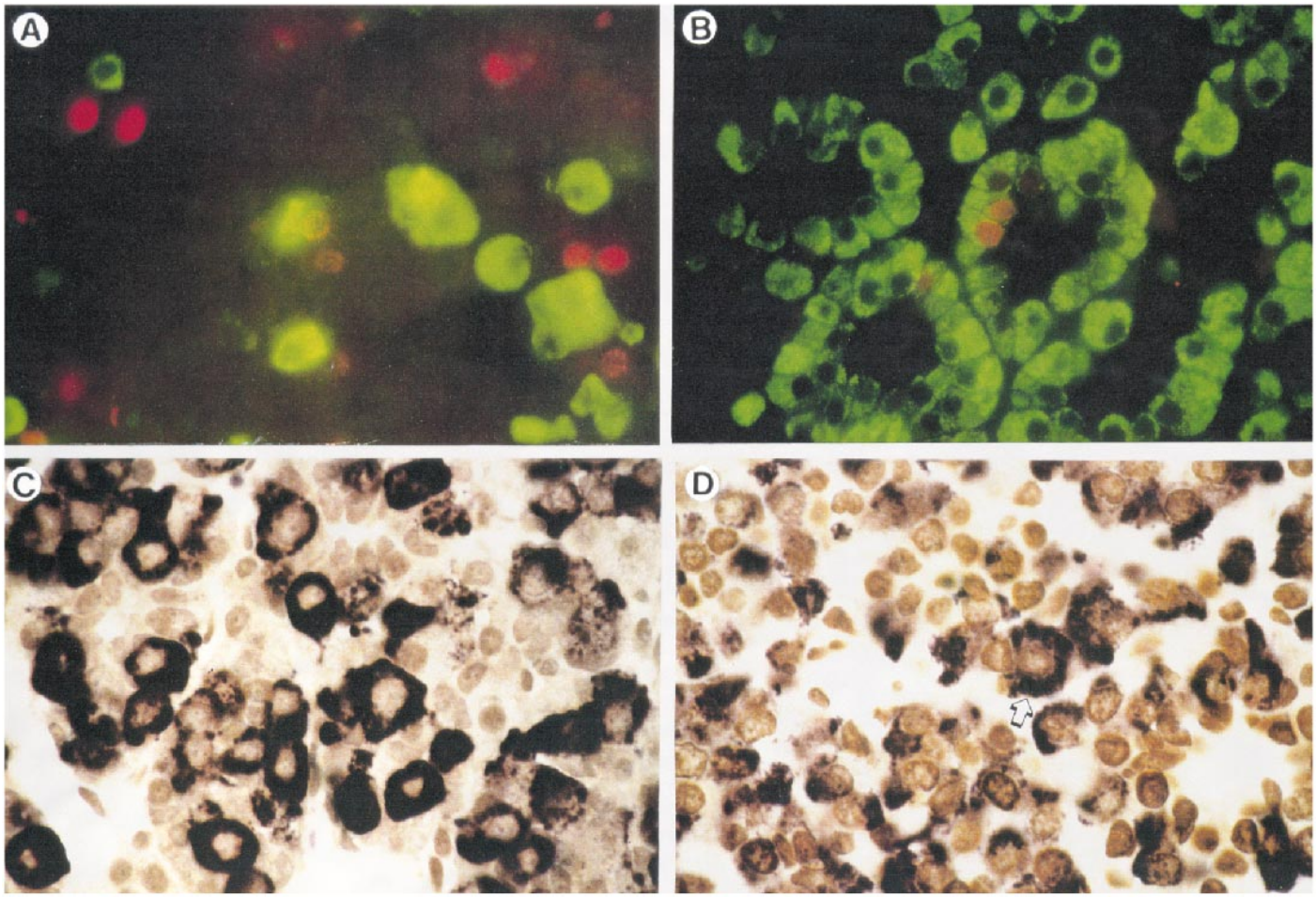

FIG. 4. **A**, **B**) Photomicrographs of pituitary gland tissue sections obtained from incubating hens nest-deprived for 3 days (**A**) or 7 days (**B**) dual labeled to locate BrdU-containing nuclei (red) and GH in the cytoplasm (green). Hens nest-deprived for 3 days received a BrdU injection 14 h before being killed while those nest-deprived for 7 days were given two injections of BrdU on Day 3 and Day 6. Note that BrdU and GH are not colocalized in **A** but are present in the same cell in **B**. **C**, **D**) Photomicrographs of midsagittal sections of the anterior pituitary gland of incubating turkey hens nest deprived for 0 days (**C**) or 4 days (**D**). The tissue sections are dual labeled to locate PRL-ir cells (black) and nuclear DNA fragmentation (brown). The arrow in **D** highlights a PRL-ir cell showing DNA fragmentation. Original magnification ×1000.

proportion of anterior pituitary cells that secrete PRL and a comparable decrease in the percentage of cells that release GH [15]. Furthermore, separating lactating female rats from their pups for 4 days induced a reciprocal shift in the proportion of GH and PRL cells back to the levels found in virgin females [5].

Recruitment of somatotrophs in the pituitary gland caudal lobe in response to nest-deprivation of turkey hens may result from cell division of existing GH-ir cells, and/or transdifferentiation of lactotrophs or other pituicytes. In order to test the first possibility, we administered BrdU, a thymidine analogue used to identify mitotic cells in the S-phase of cell division [16], to the nest-deprived turkey hens. There are no previous reports that show proliferation of turkey pituicytes in adults. In incubating turkey hens (nest-deprived for 0 days) and in laying hens, we did not find proliferation of pituicytes based on the complete absence of BrdU-ir nuclei. Following nest-deprivation for 1 or more days, an increased proliferative activity was noticed in both cephalic and caudal lobes of the pituitary gland. Nest-deprivation for 7 days resulted in the highest frequency of proliferating cells both in the cephalic and caudal lobes probably due to administration of two injections of BrdU to this group (on Days 3 and 6). Dual labeling procedures revealed that somatotrophs do not colocalize BrdU, suggesting that somatotrophs in the caudal lobe do not undergo cell division following nest-deprivation for 1, 2, 3, or 4 days. In the hens nest-deprived for 7 days, however, all the proliferating nuclei in the caudal lobe were colocalized with GH suggesting that 1) cells in the caudal lobe that undergo mitosis ultimately differentiate to somatotrophs, 2) somatotrophs undergo mitosis only after 6 days of nest-deprivation, and 3) kinetics of BrdU-labeling is different for somatotrophs compared with nonsomatotrophs.

In chickens, an increase in pituitary cell proliferative activity, as detected by increased frequency of nuclei containing proliferating cell nuclear antigen was reported in response to forced molting [17]. In rats, pituicyte proliferation rate as revealed by BrdU incorporation was found to be influenced by sex, estrous cycle, and circadian change [18]. Adrenalectomy in rats resulted in proliferation of pituitary corticotrophs and the major mechanism of corticotroph recruitment was due to differentiation of immature cells into ACTH-secreting cells [19].

It is not clear at this time what factor(s) induce proliferation of pituitary cells or differentiation of newly divided cells into GH production. In the embryonic chicken pituitary gland, corticosterone has been found to augment dif-

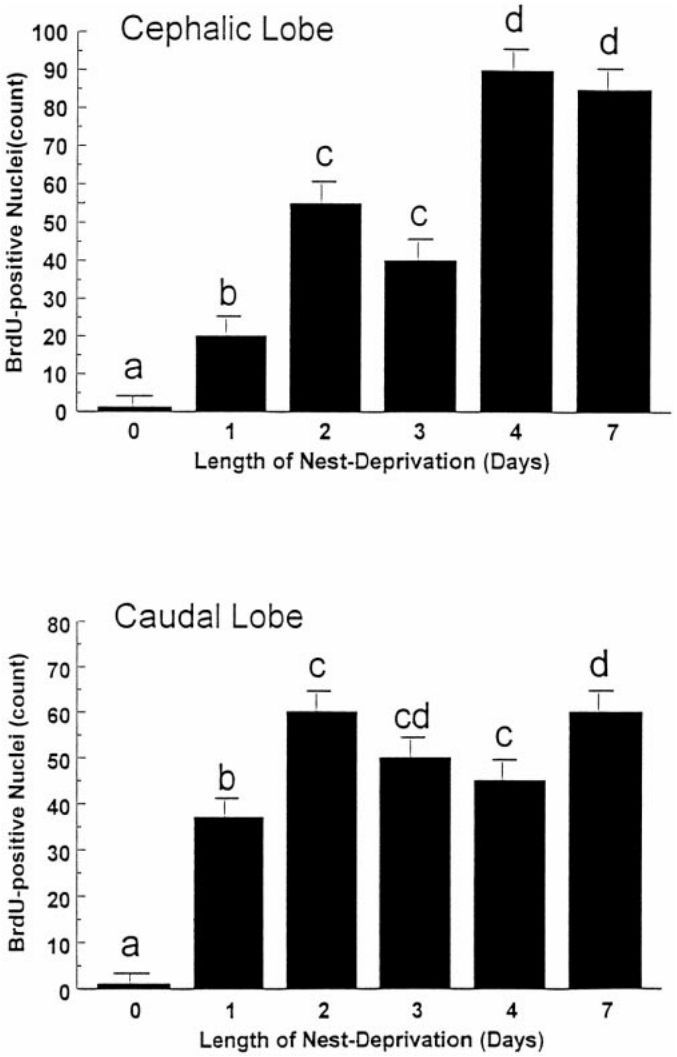

FIG. 5. Counts of proliferating nuclei in the cephalic or caudal lobes of turkey anterior pituitary gland nest-deprived for 0, 1, 2, 3, 4, and 7 days. Bromodeoxyurindine was administered before sacrifice (see *Materials and Methods*) and the pituitary tissue sections were immunostained to locate BrdU-incorporated nuclei. Counts of BrdU-positive nuclei in the cephalic and caudal lobes were obtained from tissues sections (four sections/bird; three or six birds/treatment) and compared among the treatment groups. Bars represent mean counts ± SEM. Significant differences ($P < 0.05$) are denoted by different letters.

ferentiation of GH-secreting cells [20]. Studies with mammalian pituitaries have shown that sustained stimulation of somatotrophs or corticotrophs with secretagogues result in both hypertrophy and hyperplasia of the target cells. For instance, corticotropin-releasing hormone induces hyperplasia of pituitary corticotrophs [21–23], while somatotrophs undergo hyperplasia in response to sustained stimulation by GHRH both in vivo [24] and in vitro [25]. Similarly, chronic LH-releasing hormone treatment causes hyperplasia of gonadotrophs in rats [26] while stimulation of secretory activity of thyrotroph results in their hyperplasia [27].

Mammosomatotrophs were proposed as transitional cells in the bidirectional interconversion of somatotrophs and lactotrophs in the rat pituitary gland [5]. We reported earlier that there is an increased abundance of mammosomatotrophs [3] during hyperprolactinemia associated with incubation behavior in turkey hens. In the present study, nest-deprivation of incubating turkey hens for 1 or more days resulted in disappearance of mammosomatotrophs from the pituitary gland. This phenomenon is consistent with declining plasma PRL concentrations following interruption of broodiness but would argue against transdifferentiation of lactotrophs to somatotrophs through an intermediary mammosomatotroph. However, our study does not preclude the possibility that pituicytes other than lactotrophs may undergo transdifferentiation and contribute to somatotroph recruitment in the turkey pituitary caudal lobe following interruption of incubation behavior. Luteinizng hormone-containing cells found in the avian pituitary caudal lobe [28] may possibly transdifferentiate to somatotrophs as suggested by the fact that GH and LHβ mRNA have been colocalized in sheep pituitary cells [29]. Undifferentiated cells, immunonegative to all known hormones reported in the rat pituitary gland [17], may possibly serve as a source of cells for differentiation into somatotrophs in nest-deprived turkey hens.

Nest-deprivation of incubating turkey hens result in a precipitous decline in plasma PRL concentrations, and therefore we hypothesized that lactotrophs in the pituitary gland would undergo programmed cell death. Programmed cell death is a selective process of physiological cell deletion [30–33], and it is morphologically known as apoptosis [34]. The gross anatomical features of apoptosis include nuclear fragmentation, chromatin condensation, and DNA cleavage [34, 35]. We employed a TdT enzyme-mediated dUTP nick end labeling method for detection of programmed cell death in paraffin-embedded tissue sections [13] and observed an abundance of apoptotic nuclei in both the cephalic and caudal lobes of the pituitary following interruption of incubation behavior for 2 or more days. A dual staining for PRL and DNA fragmentation showed an abundance of apoptotic nuclei in PRL-ir cells (Fig. 4D) following nest-deprivation for 2 or more days, indicating that the programmed cell death is occurring in lactotrophs. In rats, a considerable number of lactotrophs, about 25% of the total pituitary cell count, undergo regression between 3 and 7 days after withdrawal of litters [6]. Lactotrophs were found to undergo autolytic degenerative changes, but not apoptosis, in response to interruption of lactation in rats [36, 37]. Following autolytic changes, the cell debris was cleared by a pituitary and systemic mononuclear phagocytic system that may involve migration of phagocytosed stellate cells from the pituitary parenchyma into the blood circulation [38]. Pituitary gland cells harvested from rats at the end of lactation were reported to have a large number of apoptotic bodies that may have possibly arisen from redundant lactotrophs [39].

Factors that may induce apoptosis in response to interruption of incubation behavior remain to be elucidated. Vasoactive intestinal peptide has been shown to regulate mitosis, differentiation, and survival of cultured sympathetic neuroblasts [40], protect thymocytes from glucocorticoid-induced apoptosis [41], and to suppress apoptosis in the ovary [42]. In addition, pituitary adenylate cyclase-activating peptide, a member of the VIP family, prevents apoptosis in cultured cerebellar granule neurons [43]. Vasoactive intestinal peptide is the most important PRL releasing factor in avian species. An increase in hypothalamic VIP content and VIP-ir neurons was found in incubating turkey hens [44]. Furthermore, VIP concentration was higher in the incubating turkey hen hypophysial portal blood compared with the laying hen [45]. Nest-deprivation of incubating turkey hens may decrease VIP secretion from the hypothalamus. Consequently, a decline in VIP concentra-

tion in the pituitary gland may trigger programmed cell death of lactotrophs, similar to apoptotic death of adrenocortical cells occurring in response to ACTH deprivation [46, 47].

In summary, interruption of incubation behavior in the turkey hen results in marked cellular changes within the pituitary gland characterized by programmed cellular death of lactotrophs and replacement in the caudal lobe by somatotrophs by the proliferation of nonsomatotrophic cells that then differentiate into somatotrophs. The reciprocal remodeling of lactotroph and somatotroph populations appears to be initiated by the removal of neural stimulation from the brood patch, but further studies are needed to determine whether the same or different neuroendocrine pathways are involved.

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