General and Comparative Endocrinology 104, 67–75 (1996)
Article No. 0142

Changes in Pituitary Somatotroph and Lactotroph Distribution in Laying and Incubating Turkey Hens

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Accepted May 21, 1996

Turkey hens can rapidly shift from a laying condition to one characterized by ovarian regression, incubation behavior, and hyperprolactinemia. Although remarkable changes occur in hormonal profiles as turkey hens pass from a laying to an incubating state, studies have not been undertaken to examine histochemical alterations of functionally relevant pituicytes in the adenohypophysis. The objective of this study was to compare the immunocytochemical changes in pituitary lactotrophs and somatotrophs in incubating turkey hens with those of egg laying hens. Based upon nest visiting and egg production records, laying and incubating hens were selected for sampling blood, pituitaries, and ovaries. Plasma prolactin (PRL) and growth hormone (GH) concentrations were determined. Sagittal pituitary sections of laying and incubating hens were immunostained using antibodies against turkey growth hormone or synthetic chicken PRL peptide. Somatotrophs were found predominantly in the caudal lobe while lactotrophs occurred only in the cephalic lobe of adenohypophysis in laying hens. In incubating hens, somatotrophs in the ventral half of the caudal lobe were replaced by lactotrophs. The sagittal area which immunostained for PRL was significantly greater while the area that immunostained for GH was less in the adenohypophysis of incubating turkey hens. Some of the lactotrophs were hypertrophied in incubating hens. The lactotrophic recruitment and hypertrophy provide a cellular basis for the hyperprolactinemia in incubating turkey hens.

Incubation behavior in domestic turkey hens (*Meleagris gallopavo*) is characterized by increased nest visiting, ovarian regression, and hyperprolactinemia. The prolactin (PRL) producing capacity of the anterior pituitary gland is hypothesized to be increased in incubating turkey hens (El Halawani and Rozenboim, 1993). The factors that allow continuous secretion of PRL in response to PRL-releasing factor(s) are (a) increased secretory capacity of individual lactotrophs, (b) hyperplasia of lactotrophs, (c) conversion of related pituicytes to lactotrophs, or (d) a combination of the above. The third possibility of transdifferentiation of somatotrophs to lactotrophs has been shown to occur in the mammalian pituitary (Frawley and Boockfor, 1991).

Growth hormone (GH), found within pituitary somatotrophs, and PRL are closely related hormones and have evolved from duplication of a common ancestor gene (Niall *et al.*, 1971). Due to the alterations in hormonal profiles that occur in laying versus incubating turkey hens, this study was designed to document possible structural changes within the adenohypophysis. It was hypothesized that an increase in the number and/or size of lactotrophs would reflect the rapid augmentation of plasma PRL concentrations in incubating turkey hens. An immunocytochemical study of lactotrophs and somatotrophs was conducted in turkey hens sampled during defined physiological states. An emphasis was placed upon (a) the distribution pattern of somatotrophs and lactotrophs within the

0016-6480/96 $18.00

adenohypophysis and (b) the relative size of lactotrophs at defined physiological states.

MATERIALS AND METHODS

Animals

Nicholas large white turkey hens were housed in eight floor pens (3.6 × 3.6 m) with trap nests (13 birds / pen; 8 trap nests / pen). Feed and water were provided *ad libitum*. A stimulatory photoperiod of 14 hr light and 10 hr dark was provided from 30 weeks of age (lights on at 3 AM). Egg production and nesting activity were recorded every 2 hr between 7 AM and 5 PM.

Selection of Hens

Laying and incubating hens ($n = 5$) were selected based on egg production and nesting activity data following the method of Porter *et al.* (1991) after some modifications. An incubating hen was defined as one that visited the nest box more than four times out of a possible six times in a day and did not lay an egg during the last 10 days prior to sacrifice. A laying hen was characterized as one which visited a nest box one or two times a day and was laying eggs during the week of sampling the hens.

Collection of Pituitaries/Ovaries

On the day of sacrifice, each hen was quickly captured, a blood sample was drawn from the ulnar vein, and the hen was taken to a necropsy room for collection of brain and pituitary as previously described (Ramesh *et al.*, 1995). Birds were anesthetized with sodium pentobarbital and perfused with 0.75% sodium chloride containing 7.5 mg / dl heparin followed by 4% paraformaldehyde solution via the heart and carotid arteries. Brains and pituitaries were removed intact from the cranium. The mediobasal hypothalamus was then blocked and incised 3-mm lateral to the midline on right and left side. The anterior, posterior, and dorsal borders of the hypothalamus were at the levels of optic chiasm, oculomotor nerve, and anterior commissure, respectively. The mediobasal hypothalamus with pituitary was postfixed in 4% paraformaldehyde solution and stored in 0.1 *M* sodium phosphate buffer (pH 7.2) at 4° until processed. The ovary was excised and weighed, and the follicular hierarchy (if present) recorded.

Tissue Processing

Pituitaries and brain tissue of laying and incubating hens were processed in a Tissue Tek VIP 1000 tissue processor (Miles Scientific, Elkhart, IN), embedded individually in paraffin and stored at 4° until sectioned. Sagittal sections of the pituitary were cut at 10 µm thickness, using a Leitz rotary microtome, and serial sections were mounted on gelatin-coated microscope slides (four sections / slide).

Immunocytochemistry

Two antibodies from avian hormones were used for immunocytochemistry. A polyclonal antibody raised in rabbit against turkey GH (Bakst *et al.*, 1993) and a mouse monoclonal antibody (VIIA2) raised against a synthetic peptide fragment of chicken PRL (Berghman *et al.*, 1992) were utilized in the indirect immunoperoxidase method as described for the Vector-ABC Elite kit (Vector Laboratories, Burlingame, CA). Tissue sections were first deparaffinized and hydrated in descending concentrations of alcohol in distilled water. Sections were treated with a methanolic solution of 3% hydrogen peroxide for 1 hr to block endogenous peroxidase activity. Nonspecific immunostaining was eliminated by treatment with either 1% normal goat serum (for GH immunostaining) or 1% normal horse serum (for PRL immunostaining) in 0.01 *M* Tris–HCl, 0.15 *M* sodium chloride (pH 7.4; TBS) containing 1% Triton X-100 for 1 hr. Slides were incubated with either PRL antibody (1:60,000) or GH antibody (1:15,000) for 36 hr at 4° in a humid chamber. After a series of washes in TBS, slides were incubated with the appropriate biotinylated secondary antibody (1:400) for 1 hr at room temperature. Slides were then washed in TBS and incubated with avidin–biotin complex (1:200) for 2 hr at room temperature. By treating the slides with 0.001% 3,3′-diaminobenzidine tetrahydrochloride and 0.003% hydrogen peroxide, a permanent brown color reaction product was produced to identify PRL or GH immunoreactivity.

Immunocytochemical Control

Prolactin and GH antibodies were preadsorbed with their respective antigens (turkey PRL or turkey GH) for 48 hr at 4° and centrifuged at 100,000*g* for 20 min. The supernatant was used in place of the primary antibody as control while immunostaining.

Image Analysis

The immunostained pituitary sections were examined using a Zeiss Axioplan microscope to compare the distribution of somatotrophs and lactotrophs in laying and incubating hens. The microscopic images of immunostained pituitary sections were captured using a videocamera and stored in a Macintosh computer. Images of pituitary sections at a low magnification (12.5×) were outlined to obtain a measurement of total sagittal area using Image (W. Rasband, National Institutes of Health, Bethesda, MD). The areas of the anterior pituitary gland that immunostained for GH (GH-ir) or PRL (PRL-ir) were similarly outlined and summated. The resulting immunoarea measurements for each hormone per bird were ascertained based upon an average computed from four midsagittal pituitary sections. In order to get a measurement of lactotroph cell size, the diameter of PRL-containing cells showing a complete cross section of their nuclei was measured under 1000× magnification. A total of 200 cells was measured in each of the five incubating and laying hens.

Hormone Analysis

Plasma hormone concentrations (ng/ml) were measured by radioimmunoassay following the methods of Proudman and Opel (1981) for PRL and Proudman (1984) for GH.

Statistical Analysis

Ovary weights, plasma PRL and GH concentrations, pituitary PRL-ir and GH-ir areas, and lactotroph cell diameter were analyzed by *t* test using SAS (SAS, 1987).

RESULTS

Ovarian Status

The ovaries of incubating hens selected by their nest-visiting behavior were completely regressed. In contrast, all laying hens had functionally active ovaries bearing a normal hierarchy of preovulatory follicles. The mean ovary weight of incubating hens (10.28 ± 4.301 g) was significantly less than that of laying hens (164.32 ± 8.420 g; $P < 0.05$).

Plasma Hormone Concentrations

Plasma PRL concentration was significantly elevated ($P < 0.01$) in incubating hens (164.30 ± 12.502 ng/ml) compared with that in laying hens (38.40 ± 8.703 ng/ml). The mean plasma GH concentrations in incubating hens (1.42 ± 0.238 ng/ml) and laying hens (1.19 ± 0.222 ng/ml) did not differ significantly ($P > 0.05$).

Immunocytochemistry

Preadsorption control. There was no immunostaining in pituitary sections when the antibodies were first preabsorbed with their respective antigen at a concentration of 0.06 μg/ml for turkey PRL and 6.67 μg/ml for turkey GH.

Photomicrographs of representative pituitary sections of incubating and laying turkey hens immunostained for GH and PRL are given in Fig. 1. The caudal lobe and a portion of the cephalic lobe are shown. In laying hens, somatotrophs were predominantly distributed in the caudal lobe and at the junction of caudal lobe and cephalic lobe indicated by an arrowhead (Fig. 1A). In contrast, lactotrophs were present in the anterior two-thirds of the cephalic lobe (Fig. 1B). The area of the anterior pituitary gland occupied with lactotrophs appeared to be greater in incubating hens. One reason for the apparent increase in total lactotroph area was the posterior movement of the border which delineated areas occupied by lactotrophs and somatotrophs, as well as the appearance of lactotrophs in the ventral region of the caudal lobe, where somatotrophs were present in laying hens (compare Fig. 1B to 1D and Fig. 1A to 1C). To quantify the apparent increase in

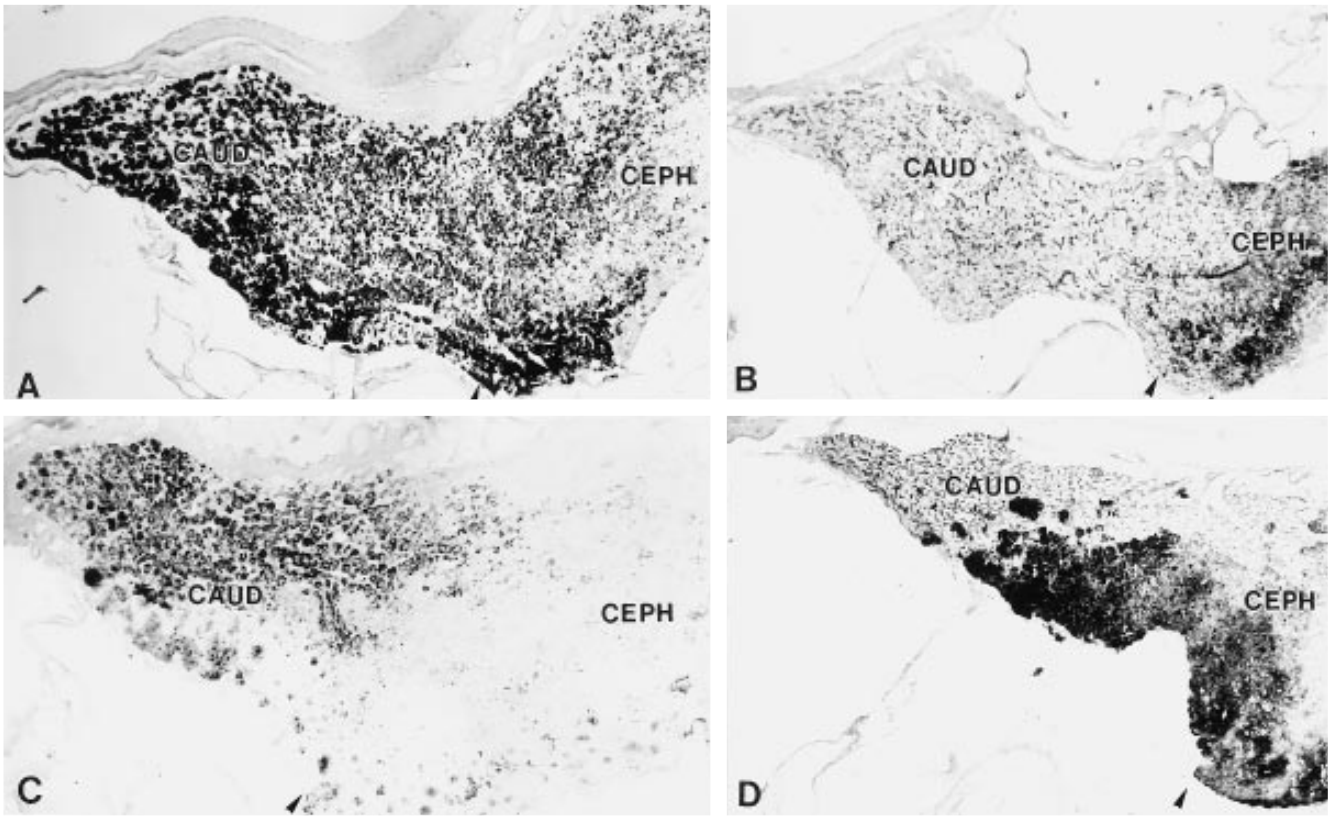

FIG. 1. Photomicrographs of midsagittal sections of the anterior pituitary gland immunostained using antibodies against chicken PRL or turkey GH. A part of the cephalic lobe (CEPH) and entire caudal lobe (CAUD) are shown at 50×, original magnification. Arrows denote the junction of cephalic and caudal lobes. (A and C) GH immunoreactivity in laying and incubating hens, respectively. (B and D) PRL immunoreactivity in laying and incubating hens, respectively.

lactotrophs, PRL-ir and GH-ir areas were determined for each of the five incubating and laying hens and compared to the total area of the adenohypophysis at midline. The PRL-ir area was found to be significantly greater ($P < 0.05$) and the GH-ir area was significantly less ($P < 0.05$) in incubating hens compared to layers (Fig. 2). The recruitment of lactotrophs in incubating hens and the characteristic presence of lactotrophs in the caudal lobe was noticed not only in the midsagittal region but in the lateral areas of the pituitary gland also (data not shown).

In addition, it was found that some of the lactotrophs appeared hypertrophied in incubating hens compared to those of laying hens (compare Fig. 3A and 3B photographed at the same magnification). The mean cell diameter of PRL-containing cells in incubating hens (12.8 ± 0.16 μm) was significantly higher compared to that in laying hens (8.5 ± 0.95 μm; Fig. 4).

Another histochemical difference observed was the immunostaining of somatotrophs throughout the anterior pituitary gland. Somatotrophs in the caudal lobe of incubating as well as laying hens were distributed individually and had dark immunoreactivity (Fig. 5A). In contrast, somatotrophs found in the cephalic lobe occurred in islets and showed less dense immunoreactivity (Fig. 5B). The area of somatotroph islets within the cephalic lobe of incubating hen pituitary did not differ from that of the laying hen.

DISCUSSION

The present study documents (a) the distribution of lactotrophs and somatotrophs in the turkey pituitary and (b) changes in the distribution of lactotrophs and

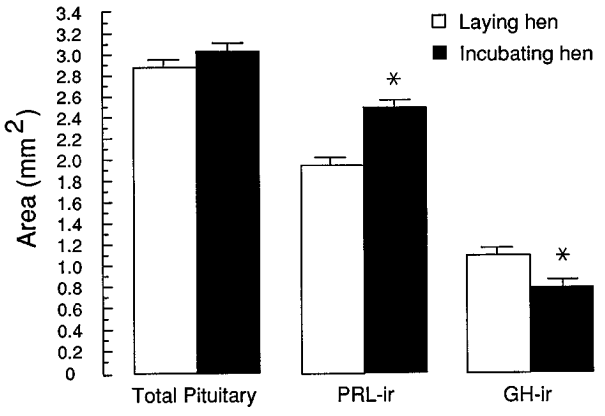

FIG. 2. Total pituitary, prolactin immunoreactive (PRL-ir) area, and growth hormone immunoreactive (GH-ir) area (mm^2) in laying and incubating hens. Midsagittal sections of pituitaries collected from laying and incubating hens were immunostained and the PRL-ir area or the GH-ir area as well as the whole pituitary area were measured by image analysis. Bars represent mean PRL-ir area or GH-ir area ± SE of five birds each. * denote significant differences between laying and incubating hens at $P < 0.05$.

somatotrophs within the pituitary as the turkey hen shifts from egg production to incubation.

The anterior pituitary gland of the turkey can be divided into cephalic and caudal lobes. A blood vessel connecting the branches of right and left internal carotid arteries was found to be a convenient marker for dividing the anterior pituitary gland into cephalic and caudal lobes. It has previously been identified as the intercarotid anastomosis (Wingstrand, 1951).

The distribution of lactotrophs and somatotrophs in laying turkey hens found in this study is similar to the localization of PRL- and GH-containing cells described in an earlier report (Ramesh *et al.*, 1995). In laying hens, lactotrophs were found to be distributed individually and as islets in the cephalic lobe. The regional localization of lactotrophs to the cephalic lobe in turkeys is similar to other avian species like pigeons (Hansen and Hansen, 1977), chickens (Jozsa *et al.*, 1979), and quails (Berghman *et al.*, 1992). Somatotrophs were found predominantly in the caudal lobe and in small numbers in the cephalic lobe in laying hens. In the caudal lobe, somatotrophs were scattered evenly and stained intensely, while in the cephalic lobe they occurred in discrete islets with much paler immunoreactivity. The occurrence of well defined, small numbers of islets composed of somatotrophs with paler staining in the cephalic lobe appears to be characteristic of the turkey pituitary. This might reflect the presence of molecular variants or isoforms of growth hormone (Houston and Goddard, 1987) and/or functional heterogeneity as found in mammalian pituitary (Perez and Hymer, 1990). The distribution of somatotrophs in the caudal lobe of the turkey pituitary was similar to other avian species, namely, pigeons (Hansen and Hansen, 1977) and chickens (Jozsa *et al.*, 1979).

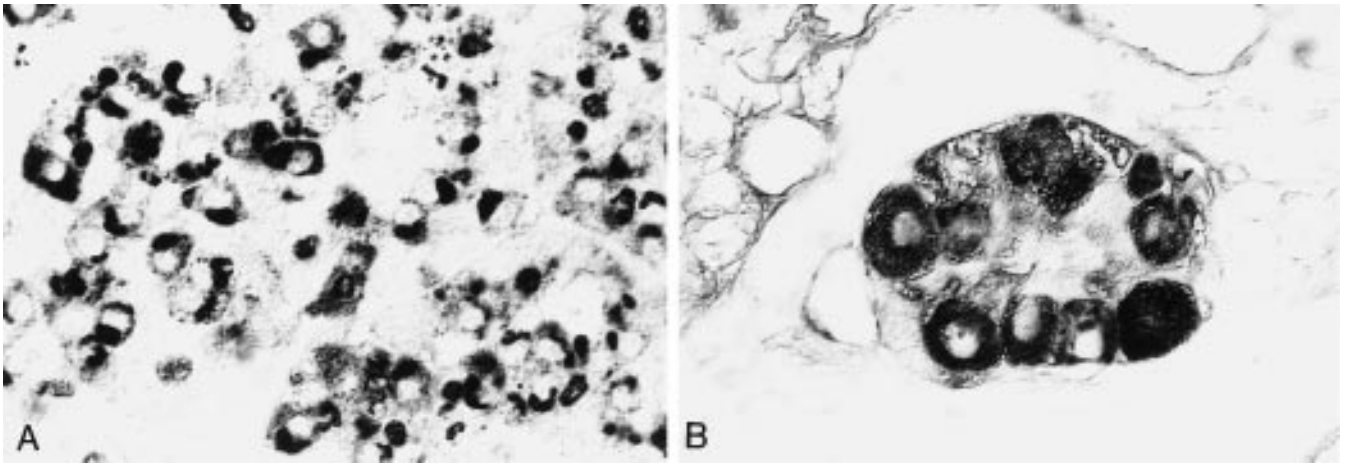

FIG. 3. Cephalic lobe of the anterior pituitary gland showing lactotrophs of laying (A) and incubating (B) hens immunostained using chicken PRL antibody. Notice hypertrophied lactotrophs in the incubating hen pituitary showing enlarged cytoplasm staining densely for prolactin. Original magnification ×1000

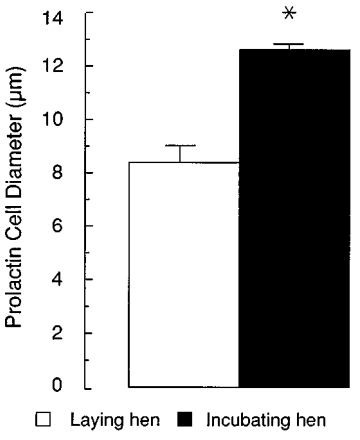

FIG. 4. Mean cell diameter (μm) of lactotrophs within the laying and incubating pituitary immunostained using antibodies against chicken PRL. Values represent the mean ± SE of five birds in each group and 200 cells were measured per bird. * indicate significant difference at $P < 0.05$.

The avian pituitary is therefore unusual among higher vertebrates due to a predominant separation of lactotrophs and somatotrophs to the cephalic and caudal lobes, respectively. This unique distribution of the cells may serve to help recognize altered regulation of phenotypically identified pituicyte populations.

The distribution of lactotrophs and somatotrophs in laying turkey hens found in the present study is also in agreement with a recent study that reported the localization of PRL secretors to the cephalic lobe and GH secretors to the caudal lobe in chicken pituitary (Lopez *et al.*, 1995). In addition, the chicken pituitary was found to contain the highest concentration of PRL mRNA and GH mRNA in the cephalic and caudal lobes, respectively (Kansaku *et al.*, 1994).

Lactotrophs and somatotrophs appear to be dynamic at the junction of cephalic and caudal lobes and along the ventral border of the caudal lobe. Somatotrophs, which were scattered throughout the caudal lobe in laying hens, were particularly absent in the ventral portion of the caudal lobe in incubating hens. Lactotrophs appeared to replace somatotrophs in these locations. This was reflected in a significantly greater PRL immunoreactive area and a significantly smaller GH immunoreactive area in incubating hens compared to laying hens (Figs. 1 and 2). The mechanism responsible for the increase in the PRL-ir area in incubating hens is not understood. One possibility is the recruitment of additional lactotrophs, particularly at the ventral half of the caudal lobe to maintain hyperprolactinemia that is characteristic of incubating hens. The lactotrophic recruitment could have resulted through proliferation of lactotrophs by mitosis and/or transdifferentiation of somatotrophs into lactotrophs.

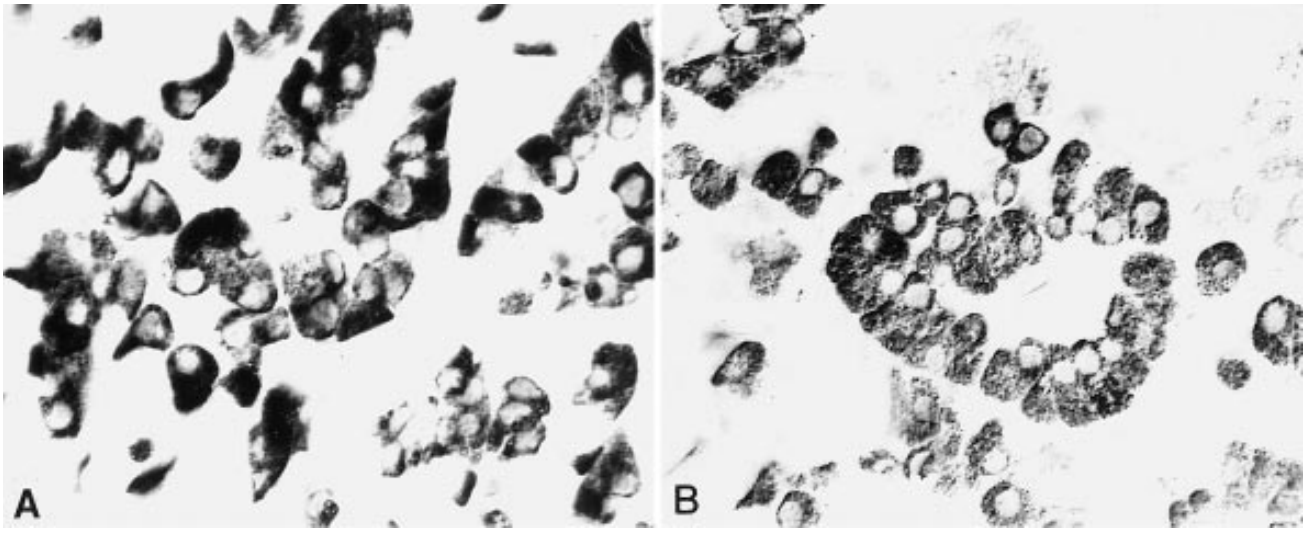

FIG. 5. Growth hormone immunoreactive pituicytes showing marked differences in immunostaining depending upon their location, (A) Caudal lobe, (B) cephalic lobe. Note darkly stained and even distribution of GH-ir cells in (A) while GH-ir cells form islets and are more lightly stained in (B). Original magnification ×1000

The regulatory mechanisms of lactotroph proliferation are poorly understood in all species. Estrogen is implicated in lactotrophic proliferation in rats by a direct action on lactotrophs (Lieberman *et al.*, 1982) and by an action mediated via the brain (Hashi *et al.*, 1995). Estrogen appears to be involved in the regulation of lactotrophs in birds since ovariectomy abolished PRL secretion in response to vasoactive intestinal peptide (VIP) administration and estrogen treatment restored the response in ovariectomized bantam hens (Macnamee *et al.*, 1986). The neural regulation of lactotroph proliferation might be important in turkeys since active immunization against VIP has been found to prevent the increase in plasma PRL levels (El Halawani *et al.*, 1995). It is not known at this time whether VIP or any other PRL-releasing factor would affect lactotroph proliferation within the adenohypophysis.

The other possible mechanism for lactotroph recruitment is transdifferentiation of somatotrophs to lactotrophs, as reported within the mammalian pituitary (Porter *et al.*, 1990). It would result in a concomitant decrease and increase in somatotrophs and lactotrophs, respectively. Mammosomatotrophs which colocalize GH and PRL are considered as intermediates in the bidirectional transdifferentiation of somatotrophs and lactotrophs (Porter *et al.*, 1990). Preliminary data in our laboratory suggest that mammosomatotrophs exist in the turkey pituitary as well (Ramesh *et al.*, 1994).

It is intriguing why lactotroph recruitment should occur at the ventral portion of the pituitary caudal lobe in incubating turkey hens. It is already known that the posterior pituitary is involved in the regulation of PRL secretion in turkeys (El Halawani *et al.*, 1992) and in rats (Murai and Ben-Jonathan, 1987). One explanation is that the caudal lobe is anatomically proximal to the posterior pituitary and there might occur transport of growth factors from the neurohypophysis into the caudal lobe to trigger the observed cellular changes. Another possibility would be a differential vascular network within the pituitary leading to selective transport of hypothalamic factors to different regions of pituitary. In the white-crowned sparrow, *Zonotrichia leucophrys gambelii*, a separate vasculature has been shown to supply the cephalic and caudal lobes of the adenohypophysis (Vitums *et al.*, 1964). A paracrine effect of other pituitary hormones like pro-opiomelanocortin (Tilemans *et al.*, 1994) could also account for selective lactotroph development in the caudal lobe.

In addition to lactotrophic recruitment, some of the lactotrophs in pituitaries of incubating hens appeared hypertrophied. The mechanisms underlying the hypertrophy of lactotrophs are not known except that chronic diethylstilbestrol treatment in rats (Phelps and Hymer, 1988) resulted in hypertrophy and hyperplasia of PRL-containing cells. Lactotroph hypertrophy suggests that there is an increase in PRL secretory capacity which also would contribute to the hyperprolactinemia. The proportion of PRL secretors were increased in the cephalic and middle region of the anterior pituitary of incubating bantam hens (Lopez *et al.*, 1996). The lactotrophic recruitment and hypertrophy in incubating hens observed in this study would partly explain the greater pituitary PRL content and PRL mRNA abundance in incubating turkey hens (Wong *et al.*, 1992) compared with that of laying hens.

It is not known at what stage in the transition from the egg laying state to incubation behavior the observed changes in pituitary lactotrophs and somatotrophs take place. Since ovine PRL administration has been shown to induce incubation behavior (Pitts *et al.*, 1994) and plasma PRL levels increase several fold at the onset of incubation behavior (Proudman and Opel, 1981), lactotrophic changes may precede the onset of incubation behavior and high levels of plasma PRL.

In summary, incubating turkey hen pituitaries showed lactotroph recruitment and hypertrophy with a concomitant decrease in somatotroph cell population. Further studies of the mechanism leading to the observed changes in lactotrophs would help in understanding the hyperprolactinemia associated with incubation behavior.

ACKNOWLEDGMENTS

The authors acknowledge the excellent technical assistance of M. Masson for processing the photomicrographs and Denise Beaudoin and Shirley Moore for radioimmunoassay of plasma hormones. This is Scientific Article A7885, Contribution 9219 of the Maryland Agricultural Experiment Station (Department of Poultry Science). This study was supported in part by U.S.D.A. Co-op Agreement No. 58-1265-2-143.

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