Changes in the Population of Proliferating Cells in Chicken Anterior Pituitary During Induced Molting: An Immunocytochemical Analysis for Proliferating Cell Nuclear Antigen

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ABSTRACT The goal of this study was to determine whether cell proliferation is increased in the anterior pituitary during an induced molting period in chickens. The anterior pituitaries were collected from: 1) laying hens before initiation of an induced molt (control hens), 2) molting hens 2 to 16 d after cessation of lay, and 3) second cycle laying hens 1 d after returning to lay. Pituitary cephalic and caudal lobes were processed for immunocytochemistry of proliferating cell nuclear antigen (PCNA), and positive cells were counted by an image analyzer using light microscopy. In cephalic and caudal lobes, a small number of positive cells were observed in controls and molting hens 2 d after cessation of lay. The frequency of PCNA-positive cells started to increase from 7 d after cessation of lay (3 d after refeeding), and a significantly higher frequency of positive cells was observed in both lobes of molting hens 13 d after cessation of lay when compared to control hens, molting hens 2 d after cessation of egg lay, and second cycle laying hens. The frequency of PCNA-positive cells in second cycle laying hens decreased to a similar level to that of control hens. These results suggest that anterior pituitary tissue may be remodeled partially by a proliferation of cells during induced molting in hens.

(Key words: anterior pituitary, cell proliferation, proliferating cell nuclear antigen, induced molting)

INTRODUCTION

During molting, the ovary and oviduct regress, and then recover by the time egg laying resumes (Decuypere and Verheyen, 1986). Artificially induced molting is effective at improving egg quality (Nordstrom, 1980; Garlich et al., 1984; Al-Batshan et al., 1994) and egg production (Lee, 1982; Garlich et al., 1984). During induced molting, circulating levels of corticosterone and thyroid hormone increase (Brake et al., 1979; Hoshino et al., 1988; Dickerman et al., 1992), whereas luteinizing hormone (LH), estrogen, and progesterone decrease (Tanabe et al., 1981; Sekimoto et al., 1987; Hoshino et al., 1988; Dickerman and Bahr, 1989). Also, the pituitary response to LH releasing hormone (LHRH) for the secretion of LH is reduced during molting (Tanabe et al., 1981; Kawashima et al., 1992). These findings suggest that the functions of pituitary, which play a central role in the control of many endocrine functions in combination with the hypothalamus, are greatly affected by molting.

Recently, we reported that the tissue of the oviduct is remodeled by the apoptotic and hydrolytic deletion of old glandular tissues and the formation of new glands during induced molting, and suggested that this tissue remodeling may be related to the improvement of egg quality in postmolt birds (Heryanto et al., 1997). If the pituitary tissue is also remodeled by newly formed glandular cells, it is possible that such remodeling may be partially responsible for the postmolt improvement of egg laying. Perek et al. (1957) found that, based on the stainability by azan staining, acidophilic (α) cells were the most common cell type and basophilic (β) cells were relatively scarce in laying hens, whereas α cells were less numerous than β cells in molting hens. However, it is unknown whether the pituitary cell proliferation is increased by the time of second laying cycle in induced molting hens. Our goal was to examine whether pituitary cell proliferation is increased during an induced molting period, which would imply that pituitary tissue is being remodeled. Currently, an antibody to proliferating cell nuclear antigen (PCNA), which appears in the cellular nucleus during S phase in a cell cycle, has become available (Coltrera and Gown, 1991). The PCNA can be used to identify proliferating cells within tissues. We detected proliferating cells in the pituitary during induced molting by immunocytochemistry for PCNA.
FIGURE 1. A and B) Sections taken from cephalic and caudal lobes of the anterior pituitary in a hen before molting treatment immunostained for proliferating cell nuclear antigen. A) cephalic lobe. B) caudal lobe. Arrow indicates an example of a cell positive for immunoreaction. Sections are counterstained with hematoxylin. C and D) Sections taken from the cephalic and caudal lobes of the anterior pituitary in a hen 13 d after cessation of laying immunostained for proliferating cell nuclear antigen. C) cephalic lobe. Arrow indicates an example of a cell positive for immunoreaction. D) Large and small arrow heads indicate examples of large and small cells, respectively, that are positive for immunoreaction. Sections are counterstained with hematoxylin.

MATERIALS AND METHODS

White Leghorn hens (approximately 550 d old) laying more than three eggs in a sequence were housed in individual cages under a daily cycle of 14 h light and 10 h dark. Molting was induced as described by Heryanto et al. (1997). Briefly, feed and water were withdrawn for 3 d. The birds were then provided with water beginning on Day 4 of treatment and thereafter. Gradual feeding was started 4 d after the cessation of egg lay. The birds ceased laying by this method usually within 7 d and resumed lay within 21 to 23 d after the cessation of lay, whereas feather loss did not occur during the entire period of this process.

Birds were killed by decapitation before molting treatment (control hens), 2, 7, 10, 13, and 16 d after cessation of egg laying (molting hens), and 1 d after resumption of laying (second cycle laying hens). Four birds were used in each group. The laying hens of control and second laying cycle were used 5 h after oviposition of the second egg in a sequence. Ovarian morphology was observed by necropsy. The anterior pituitary was fixed with 10% formalin in PBS and embedded in paraffin. Longitudinal sections of each pituitary sample (6 μm thick) were prepared and immunostained for PCNA. For immunostaining, the sections were incubated with 1% blocking reagent (casein milk) in PBS for 15 min followed by incubation with mouse monoclonal antibody to synthetic PCNA for 2 h. After washing with PBS for 15 min (thrice for 5 min), the immunoreactions on sections were detected by an avidin-biotin complex method using a mouse S-HRP immunostaining kit according to the manufacturer’s protocol. The immunoreactions for PCNA were visualized by incubating with 3′,3′ diaminobenzidine and H2O2. As a final step, sections were counter-stained with hematoxylin. Control slides were prepared in an identical manner except that the first antibody was replaced with normal mouse IgG.

The sections were examined under a light microscope, and the population of PCNA-positive cells was analyzed by an image analyzer system. The total cell number

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1Boehringer Mannheim Co., Tokyo, Japan.
2Biomeda Co., Foster City, CA 94404-8045.
3Seikagaku-kogyo Co., Tokyo, Japan.
4MacAspect, Mitani Co., Fukui, Japan.
PITUITARY CELL PROLIFERATION DURING INDUCED MOLTING

RESULTS

The PCNA-positive cells were observed in both cephalic and caudal lobes in all groups of birds and the specific immunoreactions were observed in the cellular nucleus (Figure 1; A–D). In both lobes of control birds, a small number of PCNA-positive cells were observed in both lobes, and the cells that were positive for PCNA were not uniform in shape and size (Panels A and B). In molting hens 13 d after cessation of lay, the greatest population of positive cells among each hen group were observed in both lobes (Panels C and D). Two types of positive cells, large and round cells and small and irregular cells, were observed in the caudal lobe of these hens (Panel D). The localization of PCNA-positive cells in the second cycle returned similar to the control birds (data not shown). No staining was observed in the control slides, which were incubated with normal mouse IgG in place of the first antibody.

In the cephalic lobe, the frequency of PCNA-positive cells was low in control hens and molting hens 2 d after cessation of lay, whereas the frequency of positive cells started to increase from 7 to 10 d after egg lay ceased (Figure 2). The frequency of PCNA-positive cells was greatest in molting hens 13 d after cessation of lay, and its frequency was significantly higher than that found in either control hens or molting hens 2 d after cessation of lay ($P < 0.05$). The frequency of PCNA-positive cells decreased upon resumption of egg lay (second cycle laying hens) to a level that was similar to that found before molting (control hens). The changes in frequency of PCNA-positive cells in the caudal lobe among each group of birds mimicked those described above for the cephalic lobe (Figure 3). Differences in the positive cell frequency between the cephalic and caudal lobes were not significant within each corresponding experimental hen group.

The ovary was completely regressed and devoid of any small yellow and preovulatory follicles in molting hens 7 d after cessation of lay. Small yellow follicles were present in molting hens 10 d after cessation of lay, and progressively larger follicles in hens 13 d and 16 d after cessation of lay and full hierarchy of preovulatory follicles in second cycle laying hens were observed.

DISCUSSION

The frequency of the PCNA-positive cells was increased in the anterior pituitary during the middle phase of induced molting, that is, around 20 d from the start of molt treatment (molting hens 13 d after cessation of lay). The frequencies in the cephalic and caudal lobes of these birds were, respectively, approximately 4 times and 17 times more than those of control hens. This result suggests that, in the anterior pituitary, the proliferation of cells is increased during induced molting because
PCNA appears specifically in the proliferating cells (Coltrera et al., 1991).

In both cephalic and caudal lobes, the increase in PCNA-positive cell frequency was observed 7 to 16 d after cessation of lay (3 to 12 d after refeeding) with a peak on Day 13 after laying ceased. This increase preceded the onset of the recovery of the ovary with reappearance of small yellow follicles 10 d after cessation of lay and progressive growing of these follicles thereafter. Previous workers who examined the chicken pituitary cell subpopulations by immunocytocchemistry reported that adrenocorticotropic hormone-, prolactin-, and thyroid-stimulating hormone-secreting cells (ACTH, PRL, and TSH cells, respectively) were localized in the cephalic lobe, growth hormone-secreting cells (GH cells) were in the caudal lobe, and follicle-stimulating hormone and LH secreting cells (FSH-LH cells) were in both cephalic and caudal lobes (Mikami, 1983). In chickens, GH cells were large acidophilic cells, whereas FSH-LH cells were small, narrow basophilic cells (Hodges, 1981). Therefore, we conclude that the small and large cells positive for PCNA in the caudal lobe observed in this study might be the FSH-LH and GH secretory cells, respectively. Tanabe et al. (1981) reported that, in chickens, plasma LH level and sensitivity of pituitary cells to LHRH for LH secretion were decreased during 7 d of starvation. Also, they found that LH level and pituitary sensitivity to LHRH recovered within 3 to 4 and 7 d of refeeding, respectively. These results temporally correlate with our findings that proliferation of cells in the caudal lobe increased shortly after refeeding and further suggests that these cells may have been LH secretory cells. We cannot speculate as to the types of PCNA-positive cells in the cephalic lobe that were proliferating during molting. Determination of the types of PCNA-positive cells by pituitary hormone secretory type in both the cephalic and caudal lobes during molting is clearly an important consideration.

Tsonis et al. (1988) reported that in vitro ovine pituitary cell division was stimulated by a mitogenic factor produced by the thecal-stromal layers of chicken preovulatory follicles. In chickens, PRL had a mitogenic action on the pituitary cells (Maiti and Chakraborty, 1981). The GH-releasing hormone from the hypothalamus also stimulated the mitosis of pituitary GH cells (Porter et al., 1995). These results suggest that, in hens, pituitary cells have the potency to proliferate, as observed in this study, and complex signals may be involved in the regulation of pituitary cell proliferation during induced molting.

In conclusion, our results suggest that pituitary tissue may be partially remodeled by a proliferation of cells during induced molting. This tissue remodeling may be related to the improved postmolt performance in layers.

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REFERENCES


