Prolactin effects on ultimobranchial and parathyroid glands in pigeon

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Abstract. Columba livia were injected intraperitoneally daily with ovine prolactin in a dosage of 10 IU/100 g body wt. The birds were sacrificed on 1st, 3rd, 5th, 10th and 15th day of the experiment. A progressive increase in the calcium levels was recorded between day five till day ten. After day 15, the levels decrease slightly although it is still hypercalcemic. On day five prolactin treatment produces hyperphosphatemia which progressively increases up to day ten. On day 15, the levels are almost normophosphatemic. After five days prolactin treatment the ultimobranchial gland exhibits hyperactivity. After day ten, the nuclear volume exhibits further increase and few cells are exhausted. Following 15 days prolactin treatment few completely exhausted cells and degenerating cells have also been noticed. There is an increase in nuclear volume after 15 days prolactin treatment. After day five, the nuclear volume of parathyroidal cells exhibits a decrease which progresses till day ten. Moreover, on day ten the staining response of the nuclei of parathyroidal cells decreases. Few degenerating parathyroidal cells have also been noticed on day 15.

Key words: calcium, phosphate, bird, Columba livia, Prolactin, ultimobranchial gland, parathyroid gland

Introduction

Calcium exists as a common mineral in nature but its availability to the organisms varies depending on their habit and diet. Calcium, particularly its ionic form, plays a vital role in many biological processes (Srivastav et al. 2000, 2008, Booher 2008, De Matos 2008). Deficiency as well as an overabundance of this mineral can be harmful and life-threatening. Thus, the extra-cellular calcium levels are strictly regulated by the implication of several hormonal factors, mainly calcitonin (secreted by ultimobranchial glands in non-mammals and calcitonin cells in mammals), parathyroid hormone (secreted by parathyroid glands) and vitamin D-related metabolites (Srivastav & Rani 1988, Srivastav et al. 1995, 2000, 2008, Johnston & Ivey 2006, De Matos 2008). Recently, Charoenphandhu & Krishnamra (2007) have reported that prolactin acts as a regulator of calcium homeostasis by controlling the intestinal calcium absorption.

Prolactin stimulates the secretion of specialized epithelial cells lining the crop-sac of some birds (pigeons and doves, flamingos and penguins) leading to formation of Pigeon’s milk or crop milk which is fed to the squabs (Horseman & Buntin 1995). The hormone has been reported to – (i) play an integral role in the development of migratory conditions in birds (Holberton et al. 2008) and (ii) be of an antigonadal nature in the...
reproduction of spotted munia (Bar 2006). Moreover, gonadal regression coincides with peak prolactin secretion in starlings (Dawson 2006).

Clark (1983) stated that in aquatic vertebrates, the pituitary gland was important in the regulation of blood calcium and could be of some significance in terrestrial forms as well. Several studies indicate that among many pituitary hormones, prolactin is the hypercalcemic factor in fishes (Wendelaar Bonga & Pang 1991), amphibians (Baksi et al. 1978, Srivastav & Rani 1991), reptiles (Swarup et al. 1985, Srivastav & Rani 1990; Srivastav et al. 1994), birds (Baksi et al. 1978) and rat (Robinson et al. 1975). To the best of our knowledge there exists no report regarding the effects of prolactin on the ultimobranchial and parathyroid glands of birds. Hence in this study an attempt has been made to investigate such a response in the pigeon, Columba livia.

Materials and Methods

Adult specimens of C. livia (both sexes; body wt 280-315 g) were acclimatized to the laboratory conditions for one week. Blood samples were taken from six birds before the initiation of the experiment (zero hour). The remaining birds were divided into two groups A and B and were given the following treatment for 15 days:

Group A: Birds from this group served as control and were administered daily intraperitoneally 0.1 ml of vehicle (0.8% NaCl solution)/100 g body wt.

Group B: Birds from this group were injected intraperitoneally daily with ovine prolactin (dissolved in 0.8% NaCl solution) in a dosage of 10 I.U./100 g body wt.

During the experiment the birds were fed with soaked wheat. Six specimens from each group were anaesthetized (special care was taken for any discomfort or stressful conditions to birds) with chloroform 4 h after the last injection on 1st, 3rd, 5th, 10th and 15th day of the experiment. Blood samples were collected in heparinized tubes by cardiac puncture at each experimental interval. Plasma was separated by centrifugation and analyzed for calcium (Sigma kit) and inorganic phosphate (Sigma kit) levels.

After collection of blood samples, an area of about 6 square mm containing the ultimobranchial and parathyroid glands from either side of the trachea (on the lateral side of the carotid artery) was taken out. The tissues were fixed in aqueous Bouin’s solution. The materials thus fixed were routinely dehydrated in graded series of alcohols, cleared in xylene and embedded in paraffin. The sections were cut at 6 μm and stained with hematoxylin and eosin (HE).

Nuclear volume (ultimobranchial and parathyroidal cells) indices (maximal length and maximal width) were determined with the aid of an ocular micrometer. Fifty nuclei were measured per animal. The nuclear volume was calculated as:

$$\text{Volume} = \frac{4}{3} \pi ab^2$$

where ‘a’ is the major semiaxis and ‘b’ is the minor semiaxis.

All data were presented as the mean ± S.E. of six specimens and Student’s t test was used to determine statistical significance. In all cases the experimental group was compared to its specific time control (vehicle-injected) group.

Results

Following prolactin treatment the plasma calcium levels of C. livia exhibit no change up to day 3. A progressive hypercalcemia has been recorded from day 5 till day 10 (Fig. 1). After day 15, the levels decrease slightly although it is still hypercalcemic (Fig. 1).

There is no change in the plasma phosphate levels of C. livia following prolactin treatment for 3 days. On day 5 prolactin treatment produces hyperphosphatemia which progressively increases up to day 10 (Fig. 2). On day 15, the le-
Figure 1. Plasma calcium levels of vehicle and prolactin treated pigeons. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P<0.05) between treatments.

Figure 2. Plasma phosphate levels of vehicle and prolactin treated pigeons. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P<0.05) between treatments.
vels are almost normophosphatemic (Fig. 2).

The ultimobranchial gland of the control pigeon displays the glandular parenchyma and duct-like follicles of different size dispersed in the connective tissue stroma (Fig. 3). Clusters of epithelial cells embedded in the connective tissue stroma with a rich supply of capillary network comprise the glandular parenchyma. The parenchymatous cells vary from ovoid to polygonal in shape with a sharp ovoid nucleus. The follicles may be either unistratified (Fig. 3) or pseudostratified. Their lining consists of either low columnar, cuboidal or squamous cells. The lumina of the follicles are either empty or filled with homogenous colloid-like material which is eosinophilic in nature (Fig. 3). Sometimes the lumen contains cellular debris. The follicular epithelial cells are similar in appearance with those of parenchymatous cells.

Up to day 3 following prolactin treatment the ultimobranchial gland of *C. livia* remain unchanged. Following 5 day of the prolactin treatment the gland exhibits hyperactivity which is expressed by an increase in the nuclear volume (Fig. 4) and a weak staining response of the cytoplasm of ultimobranchial cells (Fig. 5). After day 10, the nuclear volume exhibits a further increase (Fig. 4) and few cells are exhausted (Fig. 6). Following 15 days prolactin treatment few completely exhausted cells (Fig. 7) and degenerating cells have also been noticed. The nuclear volume after 15 days prolactin treatment exhibit an increase (Fig. 4).

Histologically the parathyroid gland of the control pigeon consists of parenchymal cells arranged in cords which are separated by connective tissue strands containing blood vessels (Fig. 8). The gland contains a single cell type which are oval, rounded or irregular in shape (Fig. 8). These cells possess indistinct cell boundaries. The parenchymatous cells contain little cytoplasm and a large centrally located ovoid nucleus.

**Figure 3.** Ultimobranchial gland of vehicle-injected pigeon. HE x 400.
Figure 4. Nuclear volume of ultimobranchial cells of vehicle and prolactin treated pigeons. Asterisk indicates significant differences ($P<0.05$) between treatments.

Figure 5. Ultimobranchial gland of a pigeon following five days of prolactin treatment showing decreased staining response of the cytoplasm. HE x 400.
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Figure 6. Ultimobranchial gland of a pigeon following ten days of prolactin treatment showing exhausted cells. HE x 400.

Figure 7. Completely exhausted cells in the ultimobranchial gland of a pigeon following 15 days prolactin treatment. HE x 400.

There is no change in the parathyroid gland of prolactin treated pigeons up to day 3. After day 5, the nuclear volume of parathyroidal cells exhibit a decrease which progresses till day 10 (Fig. 9). Moreover, on day 10 the staining response of the nuclei of parathyroidal cells decreases (Fig. 10). After day 15, the nuclear volume is more or less similar to that of nuclear volume of vehicle-injected birds (Fig. 9). Few degenerating cells have also been noticed on day 15 (Fig. 11).
Figure 8. Parathyroid gland of a vehicle-injected pigeon. HE x 800.

Figure 9. Nuclear volume of parathyroidal cells of vehicle and prolactin treated pigeons. Asterisk indicates significant differences (P<0.05) between treatments.
Discussion

Prolactin is effective in inducing hypercalcemia in *C. livia*. This is in conformity with the reports of Baksi et al. (1978) who have also noticed increased serum calcium levels after prolactin administration in Japanese quail. Similar effects of prolactin have also been reported for rats (Robinson et al. 1975), *Varanus flavescens*.
(Swarup et al. 1985), Natrix piscator (Srivastav & Rani 1990), Calotes versicolor (Srivastava 2002), bullfrogs (Baksi et al. 1978), anurans (Srivastav & Rani 1991) and teleosts (Pang 1981, Wendelaar Bonga & Flik 1982, 1984, Srivastav & Swarup 1985, Srivastav 2001). The hypercalcemia observed in C. livia could be attributed to the enhanced calcium absorption in the intestine and/or to the possible enhanced resorption of bone. Prolactin has been found to stimulate intestinal calcium absorption in birds (Charoenphandhu & Krishnamra 2007).

In C. livia prolactin evoked hyper-phosphatemia. Srivastav & Rani (1990) have also observed hyperphosphatemic effect of prolactin in a snake, Natrix piscator. The present study is probably the first to report such a response of prolactin from birds. The elevation of phosphate level could be attributed to the increased bone resorption and/or mobilization of phosphate from soft tissues.

The ultimobranchial gland of C. livia becomes hyperactive in response to prolactin treatment. This is evident by the increased nuclear volume and decreased staining response of the cytoplasm of ultimobranchial cells. The hyperactivity of ultimobranchial cells could be attributed to increased plasma calcium levels caused by prolactin administration. The slight decrease which has been observed in the plasma calcium level on day 15 in the present study can be linked to the hyperactivity of ultimobranchial cells which secrete increased amounts of the hypocalcemic factor (calcitonin) to counteract the prolonged hypercalcemic challenge. There are no previously published report regarding the effect of prolactin on the ultimobranchial gland of birds. Moreover, few reports are available from fish (Srivastav & Swarup 1985), amphibia (Boschwitz 1969; Srivastav & Rani 1991) and reptile (Srivastav et al. 1994).

The secretory activity of the parathyroid gland is mainly controlled by the concentration of calcium in blood (Sherwood 1968). As such, hypercalcemia suppresses the release of parathyroid hormone and renders the parathyroid gland inactive. This supports the observations recorded in the present study regarding the inactivity of parathyroid gland which is evident by the decreased nuclear volume and degeneration among the parathyroidal cells after prolonged hypercalcemic challenge by prolactin. Inactivity leading to degenerative changes in the parathyroid gland has also been reported in response to hypercalcemia in anura (Srivastav & Rani 1991; Srivastav et al. 2008), reptiles (Srivastav & Rani 1992; Srivastav et al. 1994, 2008), birds (Swarup et al. 1986-87) and mammals (Kameda 1970, Nunez et al. 1974, Swarup & Tewari 1978, Swarup & Srivastav 1979, Koyama et al. 1984, Srivastav & Rani 1988). It would be of interest to see in future whether prolactin effects the parathyroid through the stimulation of vitamin D metabolites.

Acknowledgements. The authors express their appreciation to the National Hormone and Pituitary Program, U.S.A. for the gift of prolactin.

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North-West J Zool, 4, 2008
Prolactin and endocrine glands in pigeon


Submitted: 8 May 2008

/ Accepted: 9 August 2008

North-West J Zool, 4, 2008