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Alterations in the Prolactin Cells of Stinging Catfish, *Heteropneustes fossilis* Administered with Prolactin and Maintained in Artificial Freshwater or Calcium-Deficient Freshwater

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Abstract: The present study includes the responses of plasma calcium and prolactin cells of a freshwater teleost, *Heteropneustes fossilis* injected with prolactin and maintained in artificial freshwater or calcium-deficient freshwater. Fish were divided into groups A-D. Groups A and B were kept in artificial freshwater. Groups C and D were maintained in calcium-deficient freshwater. Vehicle was administered to groups A and C. Groups B and D were injected with prolactin. Plasma calcium levels and prolactin cells were studied after 1, 3, 5, 10 and 15 days.

In group B, prolactin treatment provoked hypercalcemia from day 3 to day 5; however, the values became normocalcemic at day 10 and day 15.

A significant decrease in the nuclear volume of prolactin cells has been noticed in 5 day prolactin treated fish (group B). This response progresses till day 15. Moreover, on day 10 and day 15, depletion in the cytoplasmic granulation has been observed.

In vehicle-injected fish (group C) the plasma calcium level decreases from day 1 to day 3 (as compared to level of the fish kept in artificial freshwater). Thereafter, the level records an increase from day 5 resulting in hypercalcemia at day 10 and day 15. In prolactin treated fish (group D) the plasma calcium level shows no change up to day 3 as compared to the vehicle-injected group (group C). From day 5 to day 15, the value indicates progressive increase in plasma calcium level.

The prolactin cells of vehicle-injected fish (group C) exhibit hyperactivity on day 3 which is evident by the degranulation, hyperchromaticity of the nuclei and increased nuclear volume. Few cells are seen to degranulate completely after day 5. From day 5 to day 15, the nuclear volume is further increased. Certain cells are seen degenerating on day 10 and day 15.

The prolactin cells of prolactin treated fish (group D) have not shown any change till day 5. Between day 10 and day 15 there is a progressive decrease in the nuclear volume. Moreover, vacuolization and degeneration have also been noticed.

Keywords: Prolactin, Pituitary, Plasma calcium, Stinging catfish, Artificial freshwater, Calcium-deficient freshwater, *Heteropneustes fossilis*

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Introduction

Prolactin (PRL) is a long peptide hormone which is found in all jawed vertebrates (Saha *et al*., 2021). It is expressed in a variety of different organs and performs diverse physiological functions (Freeman *et al*., 2000; Srivastav *et al*., 2017; Dobolyi *et al*., 2020). In fish, PRL is released into circulation mainly through the definitive mass of prolactin cells present in the pituitary (Brown and Brown, 1987; Srivastav *et al*., 2017, 2021; Saha *et al*., 2021). In fishes, prolactin has been associated with many functions – (i) reproduction (Cavaco *et al*., 2003; Ozaki *et al*., 2007), (ii) parental behavior (Tacon *et al*., 2000; Pall *et al*., 2004; Cunha *et al*., 2019), (iii) migration (Onuma *et al*., 2010; Whittington and Wilson, 2013), (iv) immunomodulation (Yada *et al*., 2004), (v) egg and larval development (Kaneko *et al*., 2002; Santos *et al*., 2003), (vi) osmoregulation (Clarke and Bern, 1980; Kelly *et al*., 1999), and (vii) hypercalcemic action through inhibition of gill calcium efflux (Flik *et al*., 1994) and by promoting gill calcium influx (Anderson and van Itallie, 2009). In teleosts PRL is designated as "freshwater adopting hormone" (Sanyal and Sen, 2018). Increased calcium accretion in bones and scales of female goldfish has been reported by Takahashi *et al*. (2008).

With this background an attempt has been made in this study to investigate the effect of prolactin administration in the catfish, *Heteropneustes fossilis* maintained either in artificial freshwater or calcium-deficient freshwater. The changes induced experimentally by the prolactin in the plasma calcium levels have been correlated with the activity of prolactin cells of pituitary.

Materials and Methods

Live specimens of freshwater catfish *Heteropneustes fossilis* (both sexes) were collected locally and acclimatized to laboratory conditions for two weeks in plastic pools. For experiments fish were kept in identical glass aquaria each containing 10 L of the medium. 12 fish were kept in each aquarium. The medium was replaced on alternate days. To avoid the effects of circadian rhythm the injections were administered at the same hour of the day (between 8.00 a.m. and 9.00 a.m.). Fish were not fed 24 h before and during the experiment.

Different artificial media i.e. freshwater and calcium-deficient freshwater were prepared as follows;

(a) Artificial freshwater: Distilled water containing (in mmol/liter): $NaCl_2$.10; Na_2SO_4 0.45; KCl 0.06; $CaCl_2$ 0.8; $MgCl_2$ 0.20. pH of the solution was adjusted to 7.6 with $NaHCO_3$.

(b) Calcium-deficient freshwater: same as above without $CaCl_2$.

Adult fish *Heteropneustes fossilis* were divided into 4 numerically equal groups each containing 50 fishes. They were given following treatments:

Group A: Fish were maintained in artificial freshwater and were daily injected intraperitoneally daily with vehicle (0.1 ml of 0.6% NaCl/100 g body wt).

Group B: Fish were kept in artificial freshwater and were daily injected intraperitoneally with 0.1 mg/100 g body wt of Prolactin.

Group C: Fish were maintained in calcium-deficient freshwater and were daily injected intraperitoneally with vehicle.

Group D: Fish were kept in calcium-deficient freshwater and were daily injected intraperitoneally with 0.1 mg/100 g body wt of Prolactin.

Prolactin used in groups B and D was dissolved in 0.6% NaCl solution. Ten fish from each group were anaesthetized with MS 222 and blood samples were taken 2 h after the last injection on 1, 3, 5, 10 and 15 days of the treatment.

Blood samples were collected in heparinized tubes by sectioning of the caudal peduncle. The plasma were separated by centrifugation and analysed for calcium levels (Sigma kits). After collection of blood samples the pituitary gland along with the brain were fixed in aqueous Bouin's fluid and Bouin-Hollande fixative for histological studies. Tissues were routinely processed in graded series of alcohols, cleared in xylene and embedded in paraffin. Serial sections were cut at 6 μm. The pituitaries were stained with Herlant tetrachrome and Heidenhan's azan technique.

Nuclear (prolactin cells) indexes (maximal length and maximal width) were taken with the aid of ocular micrometer and then nuclear volume was calculated as: volume = $4/3\ \pi\ ab^2$, 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 group.

Results

(i) *Artificial Freshwater (Groups A and B)*:

No change has been noticed in the plasma calcium levels of vehicle-injected specimens (group A; Fig. 1) throughout the experiment. Following prolactin treatment (group B) the plasma calcium levels remain unaffected on day 1. From day 3 to day 5 the levels progressively increased. The values become normocalcemic at day 10 and day 15 (Fig. 1).

The histological details of the prolactin cells (Fig. 2) of vehicle-injected fish (group A) are more or less similar to those of the details given by Srivastav *et al.* (2017). These cells remain

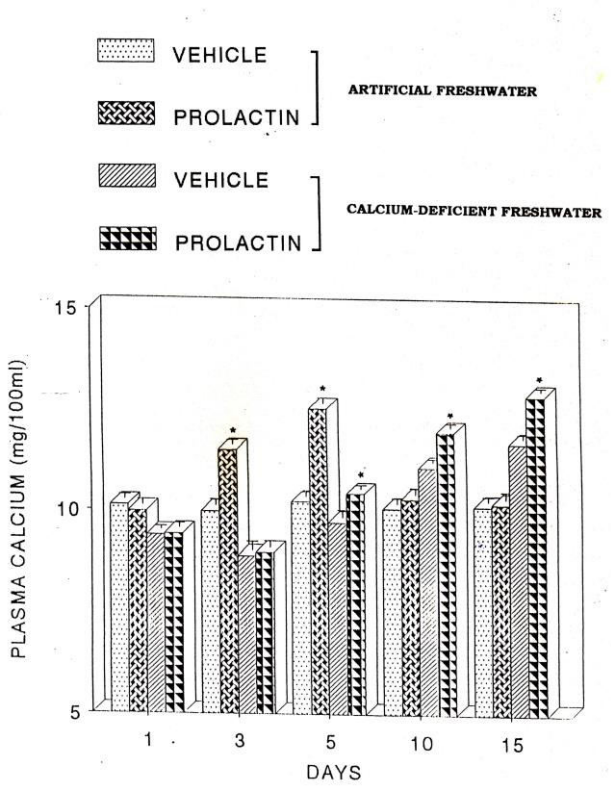

Fig. 1: Plasma calcium levels of *Heteropneustes fossilis* kept either in artificial freshwater or calcium-deficient freshwater and treated with vehicle or prolactin. Each value represents mean ± S.E. of six specimens. Asterisk indicates significant differences ($P<0.05$) with vehicle-injected specimens.

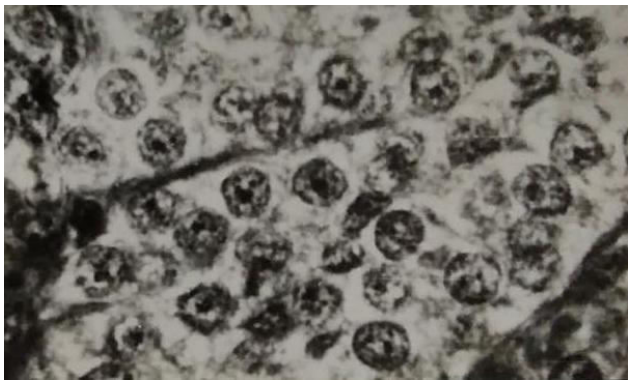

Fig. 2: Prolactin cells of 1 day vehicle-injected *Heteropneustes fossilis* maintained in artificial freshwater. Herlant tetrachrome ×800.

unaltered throughout the experiment.

A significant decrease in the nuclear volume of prolactin cells has been noticed in 5 day prolactin treated fish (group B). This response progresses till day 15 (Fig. 3). Moreover, on day 10 and day

15, a depletion in the cytoplasmic granulation has been observed.

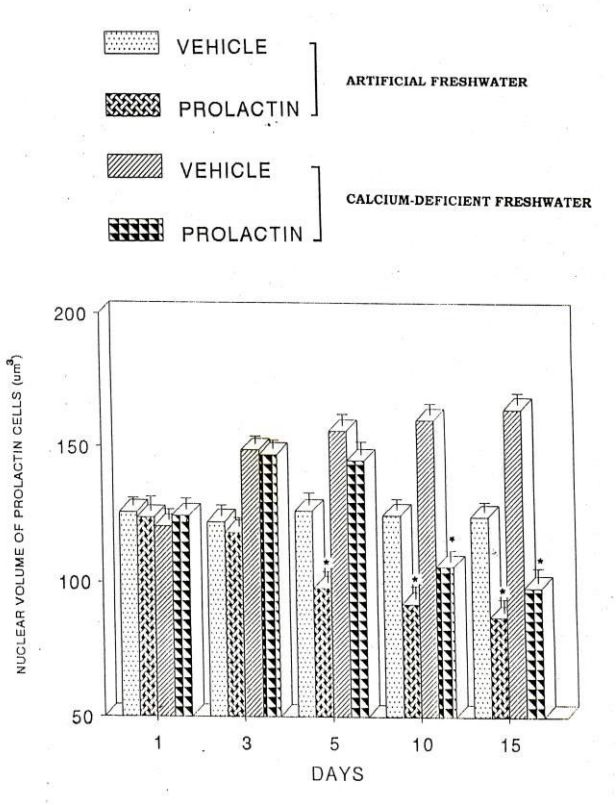

Fig. 3: Nuclear volume of prolactin cells of *Heteropneustes fossilis* kept either in artificial freshwater or calcium-deficient freshwater and treated with vehicle or prolactin. Each value represents mean ± S.E. of six specimens. Asterisk indicates significant differences ($P<0.05$) with vehicle-injected specimens.

(ii) *Calcium-Deficient Freshwater (Groups C and D)*:

In vehicle-injected fish (group C) the plasma calcium level decreases from day 1 to day 3 (as compared to level of the fish kept in artificial freshwater). Thereafter, the level records an increase from day 5 resulting in hypercalcemia at day 10 and day 15 (Fig. 1). In prolactin treated fish (group D) the plasma calcium level shows no change up to day 3 as compared to the vehicle-injected group (group C). From day 5 to day 15, the value indicates progressive increase in plasma calcium level (Fig. 1).

The prolactin cells of vehicle-injected fish (group C) exhibit hyperactivity on day 3 which is evident by the degranulation (Fig. 4), hyperchromaticity of the nuclei and increased nuclear volume (Fig. 1). Few cells are seen to degranulate completely after day 5 (Fig. 5). From day 5 to day 15, the nuclear volume is further increased (Fig. 1). Certain cells are seen degenerating on day 10 and day 15 (Fig. 6).

The prolactin cells of prolactin treated fish (group D) have not shown any change till day 5 (Fig. 1). Between day 10 and day 15 there is a progressive decrease in the nuclear volume (Fig. 1). Moreover, vacuolization and degeneration have also been noticed (Fig. 7).

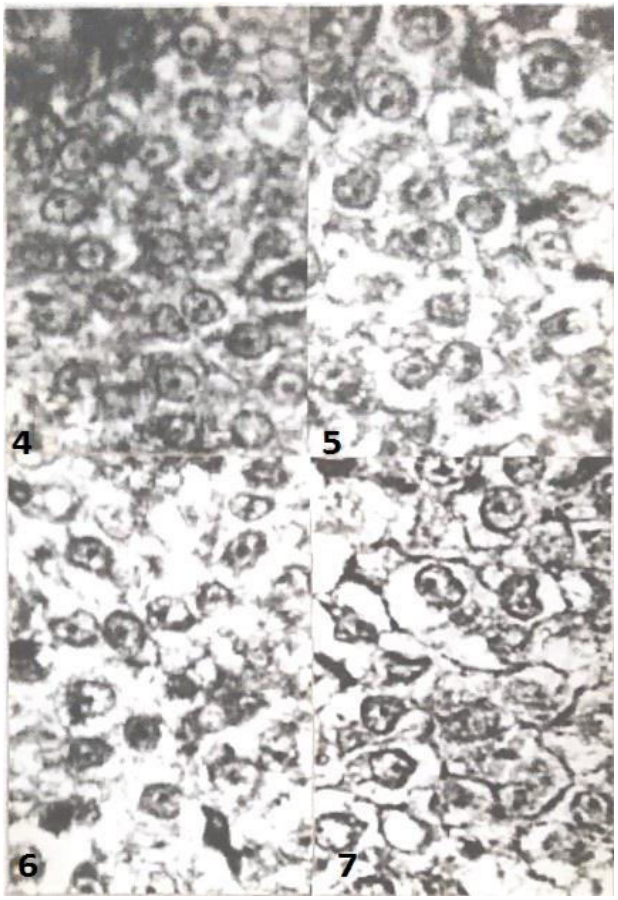

Fig. 4: Degranulation of the prolactin cells of 3 day prolactin injected fish maintained in calcium-deficient freshwater. Herlant tetrachrome x800. Fig. 5: Completely degranulated prolactin cells of 5 day vehiocle-injected fish kept in calcium-deficient freshwater. Herlant tetrachrome x800. Fig. 6: Degeneration in the prolactin cells of 15 day vehicle-injected fish maintained in calcium-deficient freshwater. Herlant tetrachrome x800. Fig. 7: Vacuolization and degeneration in prolactin cells of 15 day prolactin treated fish kept in calcium-deficient freshwater. Herlant tetrachrome x800.

Discussion

In calcium-deficient freshwater the prolactin cells of vehicle-injected *H. fossilis* exhibit degranulation and increased nuclear volume at day 3 and day 5. This can be attributed to the hypocalcemia observed in these specimens. The increased plasma calcium level noticed at day 10 and day 15 seems to be due to the increased release of prolactin from these cells. An enhanced prolactin secretion has been reported in low-calcium freshwater adapted tilapia (Wendelaar Bonga *et al.*, 1985). After acclimation to low-calcium freshwater an increased plasma calcium level has been noticed in tilapia (Wendelaar Bonga *et al.*, 1986). They have stated that this restoration of plasma calcium is most probably mediated by an enhanced production of prolactin hormone.

Prolactin treatment to the fish maintained either in artificial freshwater or calcium-deficient freshwater caused a decrease in the nuclear volume of prolactin cells. This is in conformity with the observations of earlier investigators who have also noticed a decrease in the nuclear volume of prolactin cells after inducing hypercalcemia in the fish by administration of vitamin D_3 and/or $CaCl_2$ (*Amphipnous cuchia* – Srivastav and Singh, 1989; *Heteropneustes fossilis* – Srivastav *et al.*, 1995). The present study also derives support from the studies of Wendelaar Bonga and Greven (1978) as they have reported that removal of CS causes a three-fold increase in plasma calcium which caused atrophy of prolactin cells.

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