Pituitary – gonad relationship in the male snake *Enhydris enhydris* SCHNEIDER

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Summary

The pars anterior of the pituitary gland of the estuarine snake *Enhydris enhydris* was studied in relation to the seasonal pattern of testicular activity, adopting histological techniques. Three types of basophils, type I, type II and type III, were distinguished in pars anterior of this snake adopting PAS-Orange G-Methyl Blue staining. Relying on the published literature the type I basophils (BI) are inferred as thyrotrophs, type II (BII) as FSH gonadotrophs and type III (BIII) as LH gonadotrophs, which are inferred to secrete TSH, FSH and LH, respectively. This inference is based on the observation that BI basophils underwent seasonal variation in abundance and structural features matching thyroid activity in relation to seasonal pattern of spermatogenesis, seasonal variation in BII basophils correlated directly with the seasonal spermatogenic cycle and seasonal pattern n in BIII basophils matched correlates of testicular interstitial cell activity. That the testicular activity is controlled by the pituitary gland in this snake was, further, substantiated by the regressive and degenerative changes in the seminiferous tubules. Thus, this study establishes the existence of pituitary-gonadal axis in this lizard, and there are indications that the testicular activity in this snake is under dual control of FSH and LH, as in the mammals.

***Keywords**:* Pituitary, gonad, basophils, gonadotrophs, hypophysectomy.

Introduction

Numerous studies concerning the reptilian pituitary gland have been reported since the late 1800's (see Saint Girons, 1970; Kulkarni, 1970; Licht, 1974; Licht and Pearson, 1969). Sahu and Panda (1985) discussed the reptilian pituitary system and the techniques used to identify pituitary cell types. Several investigations have correlated cyclic changes in reproductive mechanisms with cytological changes in the reptilian pituitary (Eyeson, 1970, 1971; Saint Girons, 1970; Kulkarni, 1970; Chiu and Lynn, 1971; Licht, 1982; Holmes and Ball, 1974; Del Conte, 1975; Yip and Lofts, 1976; Mohanty and Naik, 1979, 1997; Johnson and Jacob, 1984; Ferrandino et al., 2001, 2004; Jadhav and Padgaonkar, 2003; Malashetty et al., 2009).

In this study, we examined the gonadal function in relation to the pituitary gland in *Enhydris enhydris*. Our study correlates seasonal changes in testis with changes in pituitary cell types in this snake.

Materials and Methods

The snakes were collected from in and around Mumbai and acclimated to laboratory conditions prior to the experiments. Five male snakes were sacrificed each month using an overdose of sodium pentothal. The brains along with pituitaries were dissected and fixed in Bouin's fluid. After routine processing the tissues were embedded in paraffin wax, serial sections in sagital plane at 5µm thickness obtained and stained with Periodic acid Schiffs-Orange G - Methyl blue method (PAS-Orange G-Methyl Blue) as describe by Wilson and Calvin (1954). The sections were observed in the microscope for the pituitary cell types.

Hypophysectomy was performed during breeding season. The hypophysectomized and sham-operated male snakes were sacrificed at different intervals of 7, 14, 21, 28 and 36 days after the surgery (five from each time point in the case of HPX; five from the control, 36 days). Slices of testes from hypophysectomized and control animals were fixed in Bouin's fluid and processed for embedding in paraffin wax. Sections were cut at 5µm thickness and stained with hematoxylene and eosin for histological observation.

Observations

Adenohypophysis

On the basis of distribution, tinctorial affinities and morphological characteristics, six types of cells could be distinguished in pars anterior based on PAS-Orange G-Methyl Blue method. Three of them were basophils, two acidophils and one chromophobe. Since basophils are the

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ones which are concerned with the regulation of reproductive activity, further observations were limited to basophils. Three types of basophils were observed.

Basophil type I: These cells are strongly PAS-positive and in PAS-Orange G-Methyl Blue staining they are deep magenta red in color. The shape of these cells varies from almost spherical to slightly elongate. In the spherical cells the nucleus is almost centrally located (Fig. 1). Type I or B1 basophils did not show any conspicuous seasonal fluctuation in their density but indicated remarkable variation in size and cytoplasmic granulation. In July the cells started increasing in size and accumulation of granules in the cytoplasm, reaching the maximum in October (Fig. 1), the spermatogenically active phase (Fig. 3). There upon, these features decreased reaching the minimum (Fig. 2) in May at the time of complete regression of the testis (Fig. 4). Thus, its activity was parallel with spermatogenic activity of the seminiferous tubules.

Basophil type II: In PAS-Orange G-Methyl Blue staining, BII basophils are purple-colored, but otherwise almost similar in morphological features to BI basophils. These cells showed remarkable seasonal variation both in cell size and granulation. Indication of secretory activity of this cell type was parallel with the status of spermatogenic activity in the seminiferous tubules. Its size and activity increased during the recrudescent phase of the testis and reached the highest (Fig. 1) coinciding with the peak of spermatogenic activity (Fig. 3). The cells decreased in size and cytoplasmic granualation in May (Fig. 2) at the time when testis was spermatogenically quiescent (Fig. 4).

Basophil type III: These cells (BIII) were easily distinguished from BI and BII basophils on the basis of morphological and tinctorial features. They are fairly large, usually rounded or pear-shaped cells and appear to be more numerous than the other basophils. They are brick- red in color when stained with PAS-Orange G-Methyl Blue.

Effect of hypophysectomy

Hypophysectomy during the spermatogenically active phase resulted in a cessation of spermatogenesis in a time-dependent manner (Fig. 5, 6). On day 14 the seminiferous tubules showed degeneration and sloughing of the spermatocytes and accumulation of cellular debris in their lumen (Fig. 5). On day 36 the seminiferous tubules were regressed to the minimum size (Fig. 6).

Discussion

A comparison of the different cell types in the pars anterior of *Enhydris* with those described by Saint Girons (1963) in several reptilian species brings out interesting similarities. The type III basophils of *Enhydris* are comparable to LH gonadotrophs. The type II basophils are comparable to FSH gonadotrophs. Both are PAS positive. The type I basophils show close similarities to thyrotrophs.

It is difficult to ascribe specific functions to different cell types in the anterior pituitary in the absence of experimental data. Numerous attempts to identify gonadotrophs in the reptilian pituitary have produced conflicting and inconsistent results (see Licht, 1974). Two distinct gonadotropins, homologous to mammalian follicle-stimulating hormone (FSH) and luteinizing hormone (LH) been reported in amphibians, turtles, crocodiles and snakes (Licht, 1968, 1969; Licht and Pearson, 1969).

Morphology, staining properties, distribution, and seasonal cycle have suggested an increase in the number of basophils when the animal is in breeding phase. Herlant and Grignon (1961), in the turtle *Testudo mauritanica*, Saint Girons and Duguy (1962) in the snake *Vipera aspis*, Mohanty and Naik (1997) in the snake *Ptyas mucosus,* Jadhav and Padgaonkar (2003) in the snake *Cerberus rhynchops,* Ferrandino et al. (2001, 2004) in the lizards *Chalcides chalcides* and *Anguis fragilis,* and Malashetty et al. (2009) in lizard *Calotes versicolor,* identified two distinct types of gonadotrophs comparable to beta and gamma cells of mammals, in the anterior lobe of the pituitary. The activity pattern of beta cell correlated with the spermatogenic process, and the gamma cell activity matched the activity of the interstitial cells of Leydig. The beta cells described by Herlant and Grignon (1961) in the turtle *Testudo* and by Saint Girons and Duguy (1962) in the snake *Vipera,* and the basophil II described by Jadhav and Padgaonkar (2003) in *Cerberus rhynchops* are the FSH cells, and the purple basophils of *Enhydris enhydris* reported in this study have tinctorial affinities and distribution similar to these cells. Therefore, it is concluded that the beta and FSH cells described in other reptilian species and the purple basophils of *Enhydris enhydris* are homologous and may have identical function of FSH production.

The type I and type III basophils of *Enhydris* have similarities with the thyrotrophs and LH gonadotrophs, respectively, described by Saint Girons (1963).

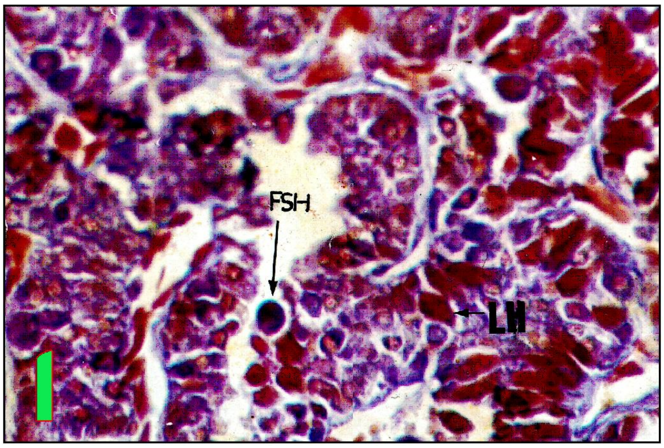

Fig.1. Photomicrograph of section of the pituitary gland during spermatogenically active phase of the snake. The unique staining patterns of FSH and LH gonadotrophs are indicated. PAS-Orange G-Methyl Blue staining method. x1000.

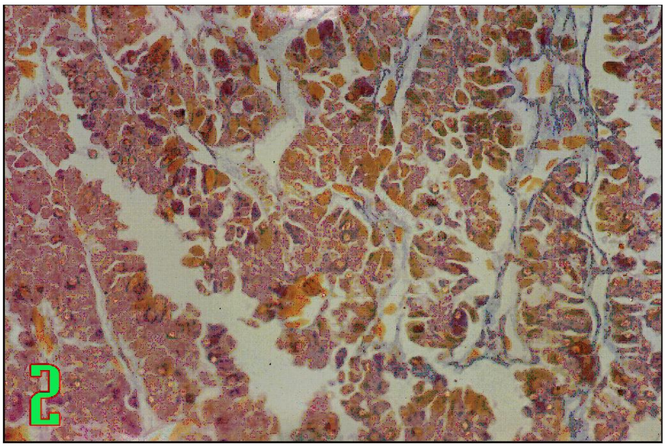

Fig.2. Photomicrograph of section of the pituitary gland during regressed phase of the testis of the snake. x1000.

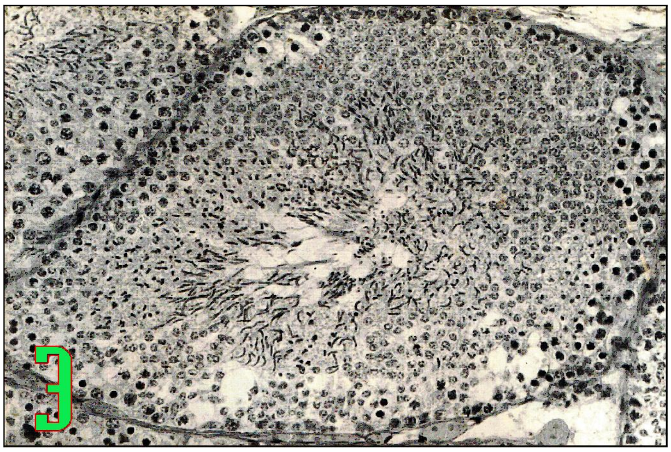

Fig.3. Photomicrograph of seminiferous tubules of the snake during spermatogenically active phase. x400.

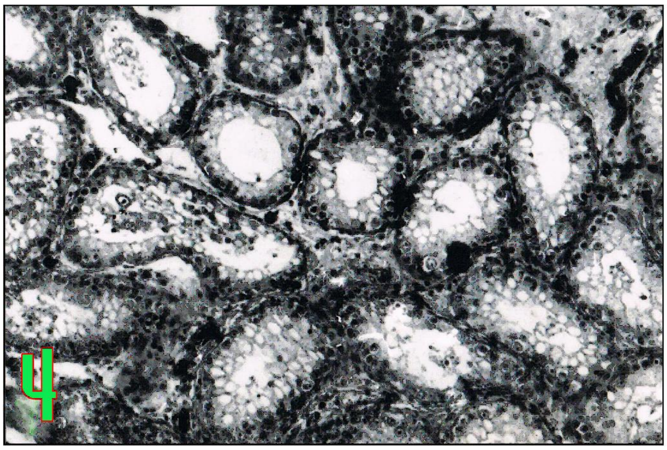

Fig.4. Photomicrograph of seminiferous tubules of the snake during the regressed phase. x400.

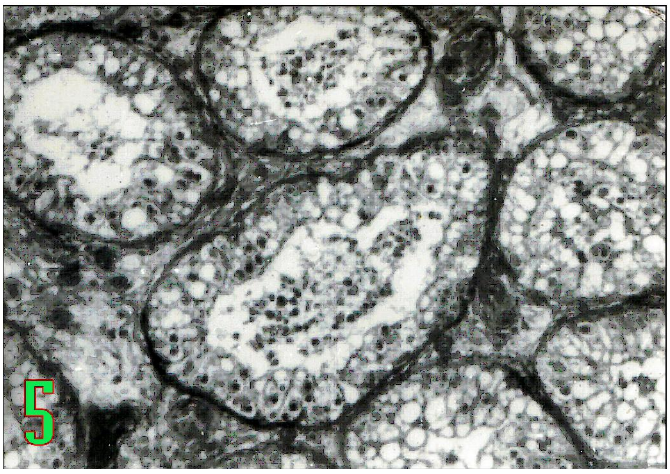

Fig.5. Photomicrograph of seminiferous tubules of the snake 14 days after hypophysectomy. x400.

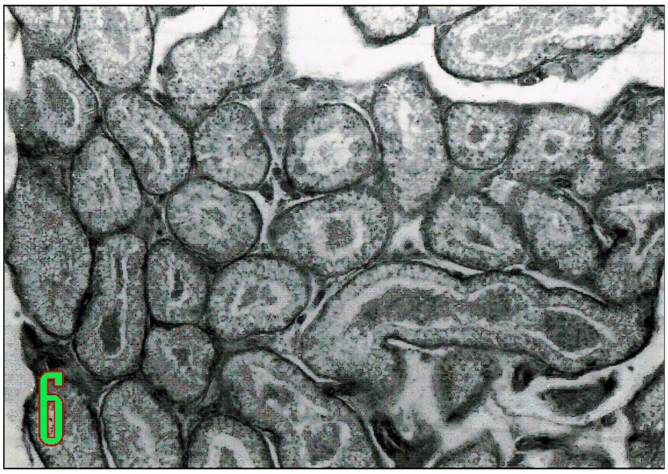

Fig.6. Photomicrograph of seminiferous tubules of the snake 36 days after hypophysectomy. x400.

In the snake *Enhydris enhydris* hypophysectomy resulted in progressive atrophy of the testis. The testicular regression was the highest, with completely collapsed tubules, 36 days following hypophysectomy. Earlier reports on hypophysectomy experiments in turtles, (Licht, 1982), lizards (Pandha and Thapliyal, 1964; Licht, 1968; Licht and Pearson, 1969; Reddy and Prasad, 1970; Eyeson, 1971; Watson, 1977) and snakes (Cieslak, 1945; Jadhav and Padgaonkar, 2003) have shown that spermatogenesis and testicular steroidogenesis are dependent on hormones produced by the pituitary.

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