Effect of mammalian gonadotropins (FSH & LH) on regressed testis of the snake *Enhydris enhydris* Schneider

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Summary

Aadministration of mammalian gonadotropins (FSH& LH) during the regressed phase of testes of the snake *Enhydris enhydris* induced spermatogenesis, and caused hypertrophy of Leydig cells. The results indicate that the testis of *Enhydris enhydris* is not refractory to gonadotropin stimulation during regressed phase of the reproductive cycle. It is further suggested that all aspects of testicular function in snakes may be accounted for by a single FSH-like gonadotropin.

Key words : Snake, regressed testes, gonadotropins, spermatogenesis

Introduction

In reptiles, due to the seasonality of reproduction, a long period of spermatogenic quiescence is recorded. The proper co-ordination of events in reproductive activity in seasonal breeders will obviously depend on the precisely timed and sequential or cyclic regulation of the pituitary gonadotropins. The long period of spermatogenic quiescence may be due to inhibition of either secretion or release of the hypophysial gonadotropins.

Although it is well established that FSH regulates gametogenesis, and LH regulates steroid hormone production in mammals, in the lower vertebrates there is controversy about the presence of two different gonadotropins, and in this contex t the reptiles are a much debated group (Licht, 1979).

Studies of the action of mammalian FSH and LH have demonstrated that both hormones bring about responses in reptiles, but FSH is more potent than LH with regard to all aspects of testicular activities (Reddy and Prasad,1970a, b; Eyeson, 1971; Lance et al., 1977, 1985; Callard et al., 1976; Tsui,1976; Tsui and Licht, 1977; Angelini et al., 1978; Licht et al., 1979; Wall, 1982K rohmer, 1986; Gaitonde and Gouder, 1985; Chandramohan and Yajurvedi,1995; Vijaykumar et al., 2002; Edward et al., 2004; Yajurvedi and Menon, 2005; Jadhav and Padgaonkar, 2010a, b).

Results from studies on the impact of mammalian hormones can have important implication for understanding the reproductive physiology of reptiles. Hence, in the present study the action of mammalian gonadotropins during the regressed phase of spermatogenesis in the snake ***Enhydris enhydris*** has been investigated.

Materials and Methods

Adult *Enhydris enhydris* males were collected from areas in and around Mumbai coast during May (regressed phase) and acclimated to laboratory condition for a week prior to the experiments. Snakes in the experimental group were given daily intramuscular injection (1IU/animal/day) of combination of FSH and LH (Pergonal-Menotropins, Serono; Switz erland: Batch No. 03 301014) for 14 (E1), 28 (E2) and 42 (E3) days. Snakes in the control group (C) received 0.01ml of saline through the same route. The animals in the experimental and control groups were sacrificed 24 hr after the last injection, by overdose of sodium pentathal. The testes from the both sides were carefully dissected out and blotted free of mucus on a blotting paper. The tissues were fixed in Bouin's fluid for 24 hr, embedded in paraffin wax, transversely cut at 5µm thickness and stained with hematoxylin and phloxine for microscopic examination.

Observations

Treatment of mammalian FSH and LH for 14, 28 and 42 days resulted in marked increase in the diameter of the seminiferous tubules. The cells of the seminiferous epithelium of the treated animals showed hypertrophy and hyperplasia where as in the control animals there was only a single layer of seminioferous epithelium. Moreover, the cell population in the seminiferous tubules of the experimental animals increased considerably. However,

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Table 1. Effect of administration of FSH and LH on the diameter of the seminiferous tubules of the snake *Enhydris enhydris*

	Diameter (μm) after 14 days treatment	Diameter (μm) after 28 days treatment	Diameter (μm) after 42 days treatment
Control	67.34±1.54	70.61±1.02	72.43±0.37
Experimental	75.73±2.65	79.35±0.03	85.33±1.36

Data represent mean values of data collected from five animals /group.

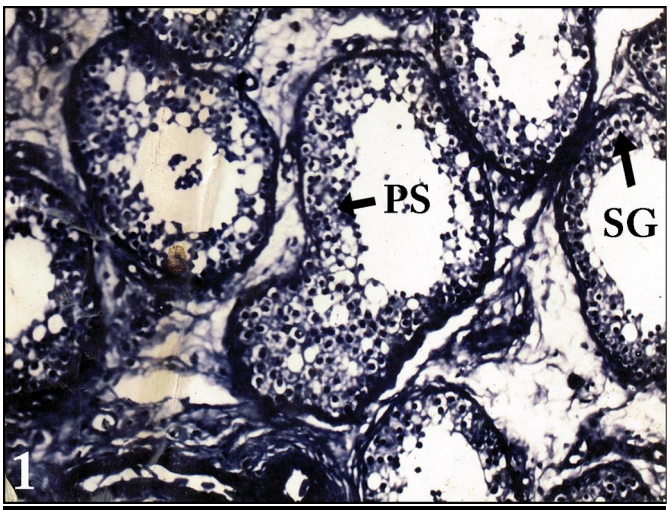

Fig.1. Cross section of the testis of a snake after 42 days of FSH and LH administration. Note the formation of primary spermatocytes. x400. SG, spermatogonia; PS, primary spermatocytes. x - 400.

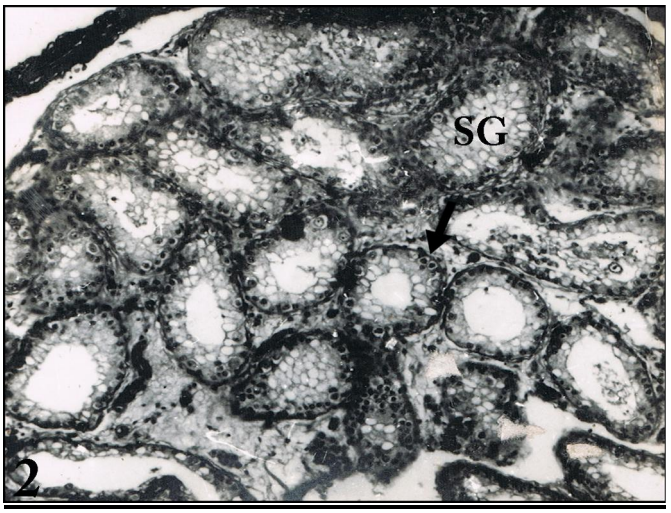

Fig. 2. Cross section of the testis of a control snake during regressed phase of the reproductive cycle. x400. SG, spermatogonia

the process of spermatogenesis in the treated animals did not proceed beyond the formation of primary spermatocytes (Fig.1, 2; Table 1). The stimulation of testes was duration-dependent; the animals treated for 42 days showed the maximum development, and lumen appeared in the seminiferous tubules.

The interstitial cells of the control and treated snakes did not show any difference.

Discussion

The general spectrum of actions and potencies of mammalian gonadotropins in the snake *Enhydris enhydris* appears to be similar to those observed for these two hormones in males of the several species of lizards such as *Anolis carolinensis* (Licht and Pearson ,1969; Licht and Stockell-Hartee, 1971; Licht and Papkoff, 1971); *Leiolopisma laterale* (Jones, 1973) and *Lacerta s. sicula* (Angelini et al., 1978). Further, administration of pregnant mare's serum gonadotropin (PMSG), FSH and human chorionic gonadotropin (hCG) to *Mabuya carinata* during regressed phase of the reproductive cycle induced spermatogenesis, spermiogenesis and hypertrophy of Leydig cells, and the result indicated that the testis of *M. carinata* is not refractory to gonadotropin stimulation during regressed phase of the reproductive cycle (Chandramohan and Yajurvedi, 1995). Also, in the same species, administration of FSH during early recrudescence phase of the reproductive cycle caused stimulation of spermatogenic and steroidogenic activities of the testis, as shown by a significant increase in the mean number of spermatogonia, primary spermatocytes, spermatids and serum level of testosterone. However, the progress of spermatogenesis beyond primary spermatocyte stage was impaired due to inhibition of gonadotropin-induced steroidogenic activity (Yajurvedi and Menon, 2005). Further, administration of purified mammalian FSH to hypophysectomized *Agama agama* caused maintenance of spermatogenesis while LH did not. However, LH acted to maintain the Leydig cells and thus provided for maintenance of the epididymis (Eyeson, 1971). Furthermore, in *Cnemidophorus tigris* administration of ovine FSH and LH resulted in production of androgens, indicating that the testis responds to both the gonadotropins (Tsui, 1976; Tsui and Licht, 1977). Iintra-testicular administration of gonadotropins (FSH and LH) produced increase of testicular and plasma androgen levels in the lizard *Uromastix hardwickii* but the increase was markedly greater in the FSH-treated lizards than LH-

treated ones (Arslan et al., 1981). Histological and histochemical analysis of epididymis of Indian wall lizard *Hemidactylus flaviviridis* revealed FSH stimulation of growth and secretory activity of testis (Prasad and Sanyal, 1969; Reddy and Prasad, 1970a, b; Haider and Rai, 1987). Administration of gonadotropins (PMSG, hCG and PMSG+hCG) to male *Calotes versicolor* during the non-breeding phase resulted in increased weight of testis and increase in diameter of seminiferous tubules and increase of spermatogonia, primary and secondary spermatocytes and spermatids. The results suggested that mammalian extra-pituitary gonadotropins also possess the potency to stimulate spermatogenesis and steroidogenesis in reptilian testis. However, the spermatogenesis was not complete since no spermatozoa were observed in the lumen of the seminiferous tubule (Sonar and Patil, 1994; Vijaykumar et al., 2002).

In male turtles (*Chrysemys picta*) mammalian FSH and LH injections produced conflicting results between intact and hypophysectomized animals (Callard et al., 1976; Lance et al., 1977).

In alligators treatment of mammalian LH-RH caused increase in plasma testosterone in all animals by 24hr (Lance et al., 1985). Compared to LH, FSH was more potent in the alligator (Lance and Vilet, 1987; Edwards et al., 2004).

FSH is clearly more potent than LH in promoting testis growth and spermatogenic activity in the snakes; all stages of spermatogenesis can be stimulated or maintained by FSH alone. Stimulation of androgenic activity is somewhat more difficult to assess in the snake *Thamnophis* and turtles *Kinosternon subrubrum* and *Pseudemys scripta* than the lizards (Licht, 1972a, b). Further, studies on seasonal effects of mammalian gonadotropins (FSH and LH) on plasma androgen levels in the male water snake *Nerodia sipedon* show that FSH stimulates testosterone production in late summer / fall, whereas LH stimulates testosterone production in the spring, but FSH was not stimulatory at this time (Well, 1982). In the immature snake *Nerodia sipedon* mammalian gonadotropins have stimulatory effect in testicular activity (Krohmer, 1986). Administration of FSH and hCG alone did not show stimulatory effect in the testes of the snake *Enhydris enhydris* (Jadhav and Padgaonkar, 2010a, b). However, in the present work FSH is more potent than LH in promoting testicular androgenesis. If at all LH is active in the snake, it appears to act like a low dose FSH in stimulating initial stages of spermatogenesis rather than being specific for stimulation of interstitial cells. Further, the snakes, as a group, have undergone major divergence in gonadotropins structure such that they may possess only a single gonadotropic molecule that bears relatively little resemblance to either FSH or LH of its ancestors (Licht et al., 1979).

On the basis of the observations on the gonadal cycle, and interpretations there-of, mammalian FSH should be stimulating spermatogenesis, and LH should be relatively specific for interstitial cells. The results of the present study clearly do not support this hypothesis. The possibility of evolutionary changes in the actions of hormones must always be considered in interpreting results with heterologous molecules. For example, the mammalian FSH molecule may possess activities normally divided between two separate hormones in reptiles. On the other hand, the presence of two separate gonadotropins is not necessarily required to explain the apparent dissociation between the two activities in the snake testis.

The present observations suggest that although mammalian FSH and LH have important differences in their biological activities, mammalian hormones are not always fully active in non-mammalian species. There must be phylogenetic specificity of the various gonadotropins for binding sites in the target tissue. Therefore, it may be concluded that each gonadotropin may not play the same physiological role in all vertebrates due to species-specificity.

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