Effect of human chorionic gonadotropin (hCG) on the regressed testis of the snake *Cerberus rynchops* Schneider

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Summary

Administration of human chorionic gonadotropin (hCG) (50 IU/Day) to the sexually quiescent snake *Cerberus rhynchops* for 14 and 28 days had no stimulatory effect on the seminiferous tubules of the regressed testes, but had a stimulatory effect in the epididymis. However, the testes of the animals treated for 28 days showed accumulation of lipid, without any spermatogenic activity in the seminiferous tubules.

Key words: Snake, testes, hCG, gonadotropin

Introduction

Mammalian FSH is capable of stimulating both spermatogenesis and steroidogenesis in squamate reptiles. Mammalian LH, possessing a similar qualitative effect, is much less potent (Licht and Pearson, 1969; Eyeson, 1971; Licht and Papkoff, 1971; Licht, 1972a, b, 1974; Lance and Vilet, 1987; Vijaykumar et al., 2002). Further, FSH alone appears capable of stimulating both gametogenesis and androgenesis. LH tends to act like a low dose FSH, and does not differently affect steroidogenesis (for review, see Licht, 1972a, b). These observations suggest that at least in squamate reptiles gonadal function might be regulated by only a single gonadotropin or a gonadotropin complex that is differentiated in mammals as FSH and LH (Licht and Stockell–Hartree, 1971; Licht, 1972a; Jones, 1973; Arslan et al., 1975). It has been also shown that, like FSH, pregnant mare's serum gonadotropin (PMSG) also is capable of stimulating testis growth, spermatogenesis, and interstitial cells in the squamates, as has been shown in the spiny tailed lizard (Arslan et al., 1975; Jalali et al., 1976), and can produce increase in the testicular and plasma androgen concentrations (Arslan et al., 1977). Furthermore, combination of mammalian pituitary gonadotropins and testosterone stimulated the epididymis of Indian wall lizard (***Hemidactylus flaviviridis***) (Haider and Rai, 1987). Mammalian non-pituitary gonadotropins (PMSG and hCG) have been shown to stimulate spermatogenesis in the lizard *Calotes versicolor* (Sonar and Patil, 1994) and bring about changes in gonadal activities in snakes (Licht, 1972a) as well as turtles (Licht and Papkoff, 1972). In the alligator, mammalian luteinizing hormone-releasing hormone (LHRH) increased the plasma testosterone concentration (Lance et al., 1985; Edwards et al., 2004). Thus, a survey of literature indicates that the actions of mammalian gonadotropins in reptiles are not the same as in mammals. Therefore, this study was taken up to find the action of hCG in the sexually quiescent snake, *Cerberus rynchops*.

Materials and Methods

The experiments were carried in the snake *Cerberus rynchops* during the testicular regression phase (April-May). The snakes were collected from the suburbs of Mumbai, India (19.16° N and 72.4° E) and acclimated to the laboratory conditions of temperature and photoperiod. Male snakes of almost similar length and weight were assigned to four groups of five each. The snakes in the first two groups were used as experimental, while those in the other two groups formed the respective controls. Snakes in the experimental groups received intramuscular injection of hCG (50 IU/animal/day) (Profasi 5000, Serono, Serum International Ltd., Batch No. 4770805C11) for 14 and 28 days, respectively. Snakes in the control groups received saline (0.9% NaCl). The snakes were sacrificed 24 h after the last injection with an overdose of sodium pentathal anesthesia. The snakes were dissected and the testes and epididymes were fixed in Bouin's fluid. The tissues were embedded in paraffin wax, 5 µm thick sections obtained, stained with hematoxyline and eosin and observed in a research microscope.

Observations

The histology of the testes of snakes belonging to the experimental groups did not differ significantly from those in the control groups. The snakes treated for 28 days did not show any spermatogonial mitosis. The seminiferous tubules of the testes of these snakes

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increased slightly in diameter and had a clearly limited epithelium and an empty lumen. The interstitial cells of the testes of hCG-treated snakes contained fibroblasts, blood vessels, and only isolated individual or small aggregates of Leydig cells containing oval nuclei and a small amount of minimally eosinophilic cytoplasm (Fig. 1, 2).

The epididymal epithelial cells of the treated animals showed hypertrophy and hyperplasia (Fig. 3, 4). The epithelial lining of the ductus deferens of these snakes also showed hypertrophy (Fig. 5, 6).

Discussion

The effect of hCG administration varies in different animal groups and also in the same group under different conditions. The interstitial cells of reptiles secrete androgen (Lofts, 1969; Licht, 1972a; Jones, 1973), and the maintenance of epididymis and renal sex segment is androgen-dependent (Prasad and Sanyal, 1969). Therefore, it is probable that the increase in epididymal epithelial height reflects increased Leydig cell androgen secretion. Administration of hCG caused a significant increase in Leydig cell stimulation in *Cerberus rhynchops* after 28 days treatment. The stimulatory effect hCG brings on the interstitial cells and as well as epididymal epithelial cells, observed in the present study, has previously been reported in the spiny – tailed lizard *Uromastix harkwickii* (Arslan et al., 1975; Jalali et al., 1976). In this lizard varying doses of ovine FSH and LH, and a lizard hypophysial extract produced increase in testicular and plasma androgen levels (Arslan et al., 1981). Male

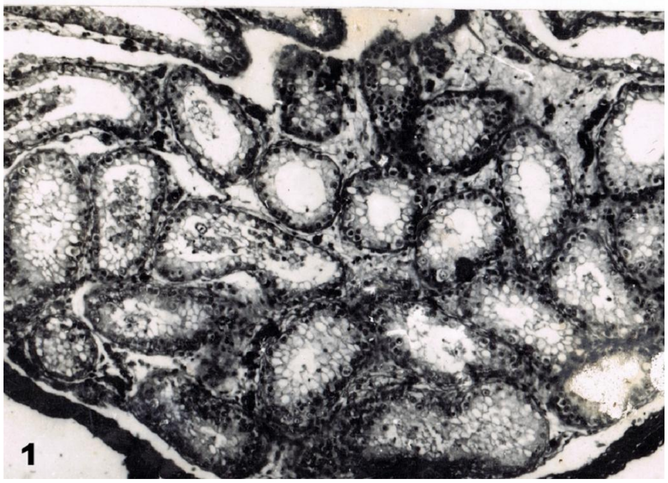

Fig. 1. Transverse section of testis of a control snake in the regressed phase. x400.

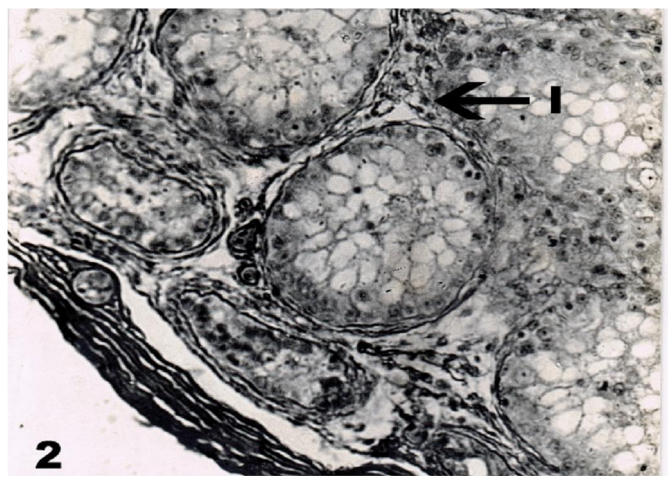

Fig. 2.Transverse section of testis of a snake after 28 days of hCG treatment. x400. I interstitial cells

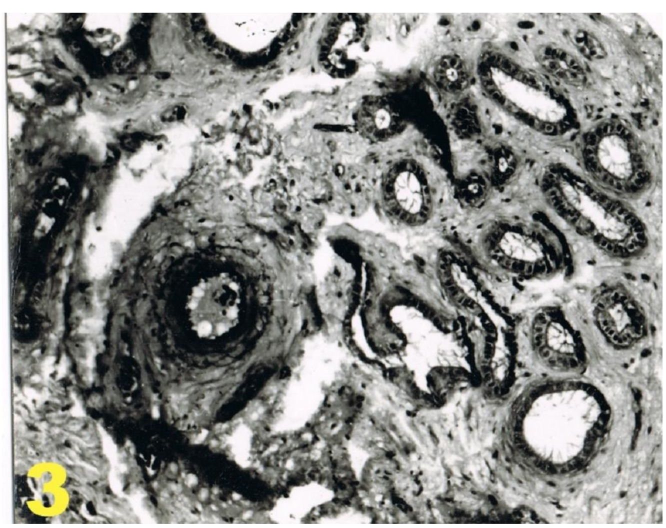

Fig. 3. Transverse section of epididymis of a control snake. x400.

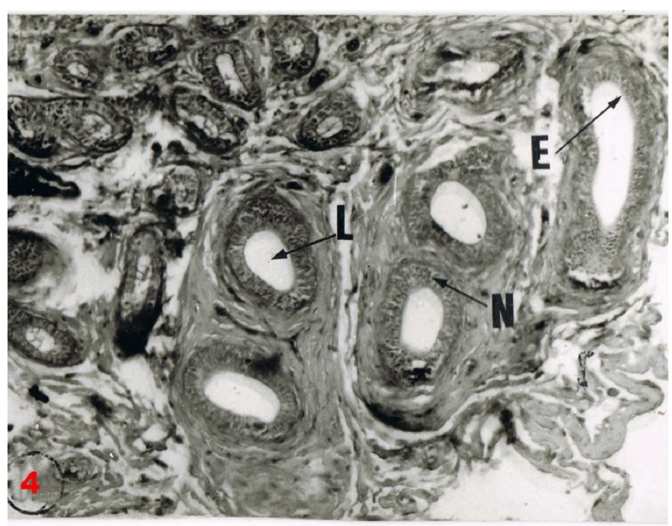

Fig. 4.Transverse section of epididymis of a snake after 28 days of hCG treatment. x400. E, epithelium; L, lumen; N, nucleus

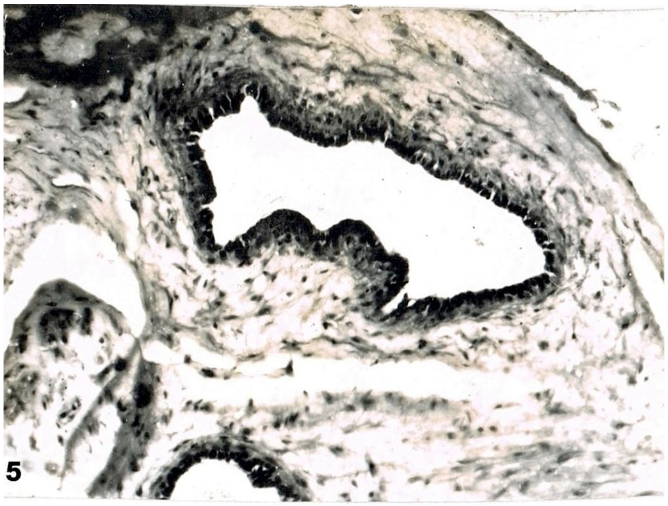

Fig. 5.Transverse section of ductus deferens of a control snake. x400.

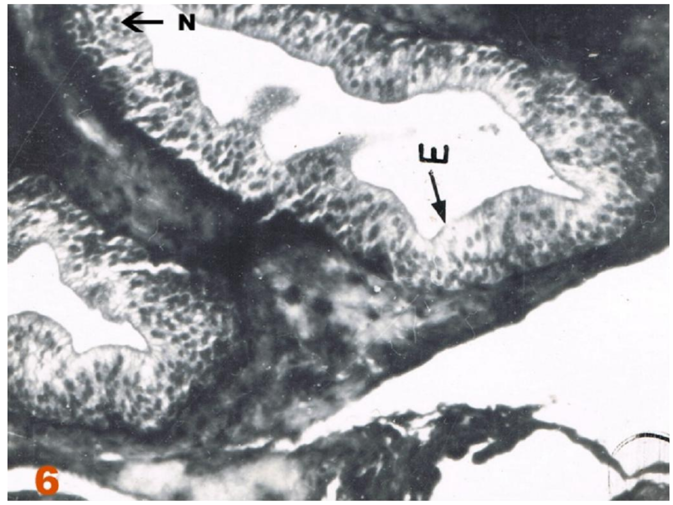

Fig.6.Transverse section of ductus deferens of a snake after 28 days of hCG treatment. x400. E, eplithelium; N, nucleus.

Leiolopisma laterale, treated with hCG, FSH, FSH+LH or PMSG, during the quiescent stage of the reproductive cycle, had increased interstitial cell number, interstitial cell hypertrophy and cytoplasmic granulation, and increased epididymal and sexual segment epithelial heights but LH had no effect (Jones, 1973). A histological and histochemical study of the epididymis of the Indian wall lizard, *Hemidactylus flaviviridis*, treated with mammalian gonadotropins and testosterone, revealed a considerable variation in the cell height and luminal diameter during the different phases of the reproductive cycle, and the investigators came to the conclusion that FSH by itself was capable of stimulating the growth and secretory activity of the epidiymis (Prasad and Sanyal, 1969; Reddy and Prasad, 1970a, b; Haider and Rai, 1987).

Treatment of exogenous gonadotropins (PMSG, hCG and PMSG+hCG) to male *Calotes versicolor* during the no-breeding phase resulted in increased weight and diameter of testis. The seminiferous tubule diameter increased and spermatids were the abundant germ cell elements. Steroidogenesis was initiated, and weight and protein content of epididymis increased. However, spermatogenesis was not complete since spermatozoa were not observed in the lumen of the seminiferous tubules (Sonar and Patil, 1994). In this lizard, combined treatment of gonadotropins (FSH+LH) was more effective than an individual gonadotropin (Vijaykumar et al., 2002). Gonadotropins induced androgen production in the interstitial cells, in vitro, of the snakes *Thamnophis sauritus* and *Thamnophis sirtalis* (Licht, 1972a).

The responses of different reptilian groups to FSH and LH differed, particularly between the turtles and the other reptiles. For example, turtles were relatively insensitivie to mammalian (ovine, bovine, rat and human) LH and hCG, whereas snakes and alligators responded similarly to mammalian FSH and LH. However, turtle testis was highly sensitive to LH preparations from many non-mammalian species, especially from the sea turtle (Tsui and Licht, 1977). In the alligator, treatment of mammalian LHRH increased plasma testosterone in blood (Lance et al., 1985; Lance and Vilet, 1987; Edwards et al., 2004).

Thus, the present study shows that hCG, when treated alone, does not bring about any major change in the spermatogenic compartment of snake testis, but the interstitial cells, epididymal epithelium and ductus deferens epithelium respond with induction/stimulation. Therefore, it is concluded that the reptiles have a unique pattern of hormonal regulation of male reproduction.

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