J Endocrinol Reprod 18 (2014) 1 : 1-6 **Original Research**

Effect of gonadotropins and gonadal steroids (17β-estradiol and testosterone) on Harderian gland porphyrin content and circulatory hormones in Indian palm squirrel *Funambulus pennanti*

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Summary

Mostly studied in the hamster, the Harderian gland (HG, an extra-orbital gland) has been shown to be capable of melatonin (MEL) synthesis and to possess steroid receptor binding sites. Sexual dimorphism and ability to respond to steroid hormones have been reported for HG of some temperate zone mammals. However, to date there is no report on functional relation of HG with pineal, pituitary and gonad in any rodent. Hence, we tested the effect of gonadotropins [FSH (10µg) + LH (10µg)] and steroids [testosterone (100µg) and 17-β estradiol (50µg)] on HG function in relation to the activities of the pineal gland and gonad in both sexes of Indian palm squirrel *Funambulus pennanti* during the sexually quiescent phase. In general, squirrels treated with gonadotropins and gonadal steroids showed a significant increase in gonadal weight and decrease in pineal weight and plasma melatonin level in LH- and testosterone-treated groups only. There was no change in the weight of HG on FSH treatment. Porphyrin content of HG decreased after testosterone and estradiol treatment. Pineal gland weight invariably decreased after treatment with gonadotropins and testosterone. Plasma testosterone and estradiol levels increased but melatonin level decreased in all treated groups. Our data suggest that in this tropical rodent gonadotropins have an indirect effect on HG and that gonadal steroids have significant effect on HG through the influence on gonadal and pineal functions.

***Key Words*:** Gonadotropins, Harderian gland, melatonin, pineal, rodent.

Introduction

The Harderian glands (HGs) of Syrian hamster exhibit marked sexual differences in cell types and porphyrin production (Chieffi et al., 1996). The HGs of male hamster have two types of secretory cells, type I filled with minute lipid droplets and type II filled with large lipid droplets, while the HGs of female hamster consists of a single secretory cell, type I, with large intraluminal deposits of porphyrin and filled with minute lipid droplets (Vilchis, 1989; Payne, 1994; Coto-Montes et al., 2001). The function of HGs is regulated by, besides androgen (Menéndez-Peláe et al., 1991), the pineal hormone melatonin (Dubey and Haldar, 1997). Because of the morphological and histoarchitectural differences in HGs between males and females, and since gonadotropins regulate secretion of steroid hormones it was hypothesized that gonadotropins may have a direct influence on the HGs. Also, it is possible that gonadotropins may have an indirect influence on HGs via regulating steroid hormone secretion because HG is a steroid-sensitive organ (Hoffman, 1971). In this study on the tropical rodent *Funambulus pennant,* in order to influence indirectly the HG function, secretion of steroid hormones by the gonad was stimulated indirectly by treatment with gonadotropins (FSH and LH) and a direct stimulation of testis / ovary with estradiol / testosterone treatment was performed. We selected the sexually quiescent phase of squirrels so that the effect of gonadotropins and gonadal steroids would be easily evident. Our study expounds a comprehensive evaluation of hormonal stimulation on the pineal, gonads, HG and its porphyrin content as well as circulatory levels of melatonin and steroid hormones of this rodent during the sexually quiescent phase.

Materials and methods

Maintenance of animals

Adult squirrels weighing 110+10 g were collected from vicinity of Varanasi (Lat. 25°18'N; Long. 83°01'E) and acclimated in a mammiliary to ambient environmental condition of temperature, humidity and photoperiod for two weeks prior to the experiment. The animals were housed in wire net cages (25' x 75' x 30') and had a free

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access to food consisting of soaked gram seeds (*Cicer arietinum*) and water *ad libitum*. All experiments were performed in accordance with institutional ethical practices and within the framework of guidelines for experimental animal studies (Committee for the Purpose of Control and Supervision of Experiments on Animals; CPCSEA).

Experimental groups

Sixty adult squirrels, 30 males and 30 females, weighing 110+10 g were selected at random and then divided into four groups, each consisting of 6 males and 6 females.

Group-I: Further divided into two sub groups. Group IA: consisting of 6 male and 6 female which received a daily dose of 0.1 ml of normal saline (vehicle control for gonadotropin treatment) and Group IB: consisting of 6 male and 6 female which received a daily dose of 0.1 ml of olive oil (vehicle control for steroid treatment treatment) through subcutaneous route for thirty days.

Group-II: Received 10µg FSH/100g body weight/ day for thirty days (Berndtson and Desjardins, 1974).

Group-III: Received 10µg LH/100g body weight/ day for thirty days (Berndtson and Desjardins, 1974).

Group-IV: The male squirrels received 100µg testosterone propionate/100g body weight/day for thirty days and female squirrels received 50µg 17β-estradiol benzoate / 100g body weight / day for thirty days (Menendez et al., 1990).

Follicle stimulating hormone (murine FSH), luteinizing hormone (murine LH) and steroid hormones (Testosterone propionate and 17β-oestradiol benzoate) were obtained from Sigma Chemical Company, St. Louis, Mo, USA. The gonadotropins were prepared in 0.9% normal saline and steroid hormones were prepared in olive oil. The injections were given between 11:00-11:30 AM daily.

Sample collection

Following thirty days of treatment blood was collected from all squirrels, under anesthesia, directly from the heart, in heparinized syringe. Plasma was separated out and stored at -20°C. Gonads, pineal and HGs were dissected out and weighed in a microelectrical balance (Sartorius AG, Germany). For porphyrin assay HGs were stored at -20°C.

Histology

HG was fixed in Bouin's fluid and, after routine processing, embedded in paraffin wax. Sections at 7 micron thickness were cut in a microtome (Leica Microsystems Inc., USA) and slides were stained with hematoxylin and eosin. The sections were observed in a research microscope (Leica MPV-3, Germany) and documented.

Hormone analysis

ELISA kits for melatonin (Kit-Kalab, Broma, Sweden) and estradiol/testosterone (Kit-Lecco-Diag Inc., Southfield, Michigun, USA) were used to measure the plasma levels of the hormones according to manufacturer's instructions. Porphyrin assay was performed according to Buzzell (1989).

Statistical analysis

Statistical analysis of the data was performed adopting one-way ANOVA followed by Students' Newman-Keel's tests. The differences were considered statistically significant when $p < 0.05$.

Results

HGs of control squirrels showed normal histoarchitecture with mostly dark type I cells and porphyrin crystals in dark dotted form in the cells and the lumen. HGs of estradiol-treated squirrels showed wider lumen, less dark cells and decreased porphyrin content. The HGs of testosterone-treated squirrels also showed larger lumen, more dark cells and less granular secretion (Fig. 1)

Relative HG weight did not reveal any significant effect of gonadotropins or gonadal steroid treatment except in estradiol-treated females which had decreased HG weight. However, pineal gland weight decreased significantly following administration of gonadotropins and gonadal steroids except FSH treatment in the case of female squirrels. The porphyrin content decreased to a significant level following gonadal steroid administration (Fig. 2, 4). Testis weight significantly increased in all the treatment groups. Hormone analysis showed increase of gonadal steroid levels and decrease of melatonin level following the treatments (Fig. 3, 5).

Discussion

Administration of FSH, LH and steroid hormones to *F. pennanti* during the reproductively quiescent phase caused decrease in pineal weight and plasma melatonin level in *F. pennanti* contrary to the report of increased activity of the pineal gland after administration of gonadotropins in the rat, a continuous breeder (Zweens, 1965) which suggest that gonadotropins and gonadal

Figure 1

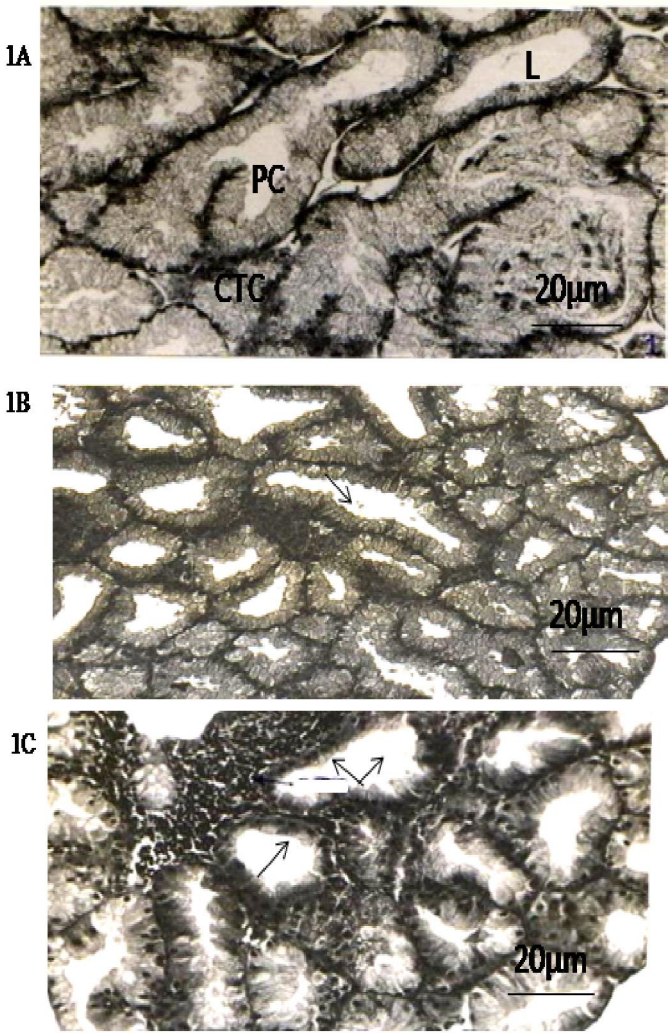

Figure 1. Histoarchitecture of Harderian gland. (A) Normal histology, having connective tissue cells (CTC), porphyrin crystals (PC) and lumen (L). (B) Histoarchitecture of HG following estradiol treatment. There is less porphyrin content. (C) histoarchitecture of HGs following testosterone treatment; note the increase in extra-cellular tissue, large lumen and cell with less granular secretion.

Figure 2

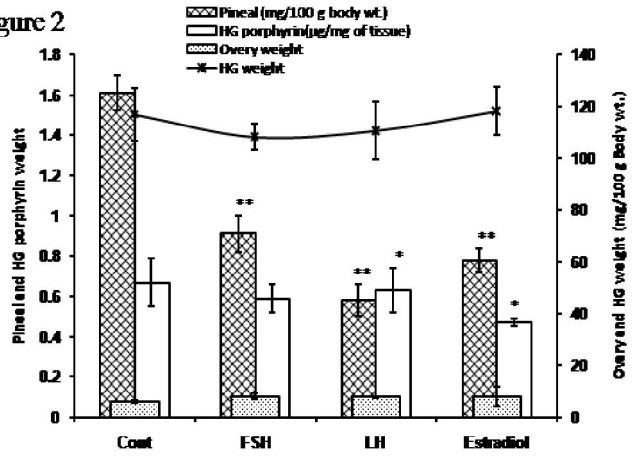

Figure 2. Effect of administration of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone on Harderian gland weight, HG porphyrin, pineal weight and testis weight of male *F. pennanti* during reproductively quiescent phase. Vertical bars represent standard error, M±SEM (N=6), Significance of difference **p*<0.05, ***p*<0.01 and ****p*<0.001.

Figure 3

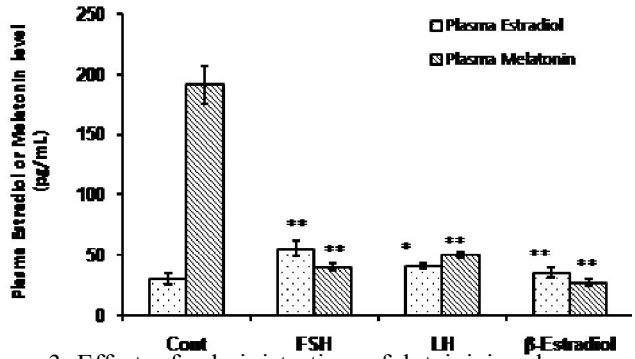

Figure 3. Effect of administration of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone on plasma melatonin and testosterone of male *F. pennanti* during reproductively quiescent phase. Vertical bars represent standard error, M± SEM (N=6). Significance of difference **p*<0.05, ***p*<0.01 and ****p*<0.001.

Figure 4

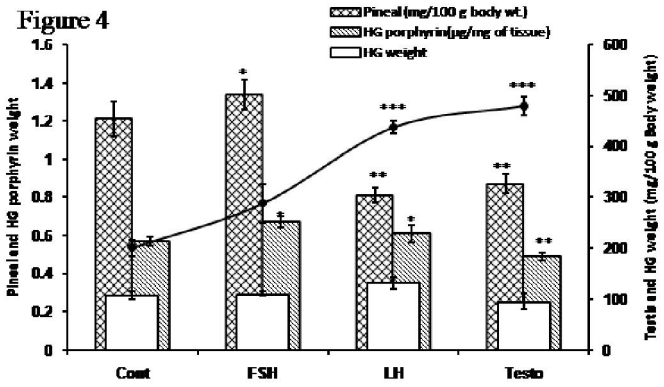

Figure 5

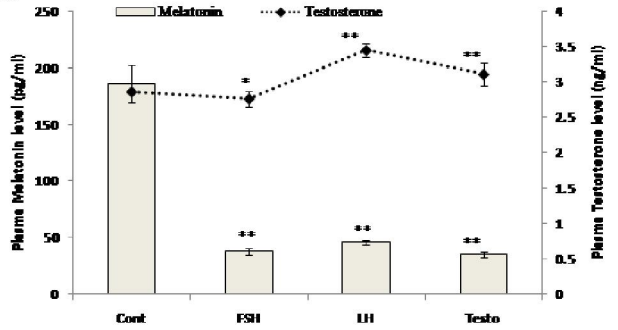

Figure 4. Effect of exogenous administration of luteinizing hormone (LH), follicle stimulating hormone (FSH) and 17-β-estradiol on Harderian weight, HG porphyrin, pineal weight and ovary weight of female *F. pennati* during reproductive inactive phase. Vertical bars represents standard error, M±SEM (N=6). Significance difference*$p<0.05$ and **$p<0.01$.

Figure5. Effect of administration of luteinizing hormone (LH), follicle stimulating hormone (FSH) and 17-β-estradiol on plasma melatonin and estradiol of male *F. pennanti* during the reproductively quiescent phase. Vertical bars represent standard error, M±SEM (N=6). Significance of difference*$p<0.05$, **$p<0.01$ and ***$p<0.001$.

hormones can inhibit active pineal function. Short term or long term treatment with FSH or LH in castrated rats resulted in a 60-80% increase of pineal hydroxyindole-o-methyl transferase activity (Cardinali, 1977). HG is an extra-pineal source of melatonin. Circulating melatonin can influence HG function (Hardeland and Uria, 1995; Haldar, 1996; Dubey and Haldar, 1997; Menéndez-Peláe, 1990). Further, injection of FSH or LH during the inactive phase increased gonadal weight, and increased plasma estradiol level which was equivalent to the level during the active phase. This suggests that low circulating levels of gonadotropins and gonadal steroids could be the reason for the gonadal inactivity though the gonads are sensitive to these hormones. Thus, administration of gonadotropins and gonadal steroids should have brought the gonads to an active state. Our data suggests that gonadotropins can inhibit pineal function indirectly.

The reported presence of estrogen receptors in the rat pineal gland indicates that a feedback mechanism does exist between ovarian estrogen and the pineal gland (Cardinali and Vacas, 1978). In the present study, administration of 17β-estradiol and testosterone caused significant reduction in pineal weight and plasma melatonin level, and increase in the gonad weight in the squirrel. These results establish an inverse relationship of the pineal gland with gonad in this diurnal rodent. The histoarchitecture of control HG showed the presence of glandular lobules with columnar cells filled with granules and smaller lumen while 17β-estradiol treatment did not affect the histoarchitecture much but the treatment of testosterone propionate caused increase of the extracellular matrix and lumen space, and the cells were having lesser granules suggesting that testosterone might have an inhibitory effect on the structure and porphyrin content of HG.

Our results suggest that unlike in the temperate zone rodent, neither gonadotropins nor steroid hormones (testosterone or estradiol) have significant effect on HG porphyrin content in this tropical rodent *F. pennanti*. This could be due to the lack of sex difference noted in porphyrin content in this squirrel contrary to the reports in hamsters (Hoffmann, 1971). Cardinali (1981), Marrufo et al. (1989) and Buzzell (1992) suggested that 5α-dihydrotestosterone administration to the female Syrian hamster directs HG to the male type and influences indolamine metabolism and porphyrin content. Further, it is also reported that hypophysectomy prevents the castration-induced increase in porphyrin content in the HG of male hamster (Buzzell, 1992). It appears that in the hamster androgen, prolactin, thyroid hormone and perhaps other hormones and environmental factors may interplay to control the porphyrin synthesis/release. Such an interaction is not evident in this tropical squirrel.

It is known that light conditions do affect the content of porphyrins in both male and female rats and hamsters (Reiter et al., 1981). Such an effect cannot be ruled out in the present context as the experiments were performed during sexually quiescent phase, i.e., during winter when the photoperiod was short (10.30 hr L and 13.30 hr D). It has been noted that gonadotropin levels are usually decreased by either blinding or exposure to short photoperiods (Brainard et al., 1984; Haldar and Dubey, 1996). Due to the removal of negative feedback inhibition, castrated hamsters have high luteinizing hormone and follicle stimulating hormone levels regardless of the photoperiod (Buzzell et al., 1989). Thus, increased gonadotropin levels of castrated hamsters did not affect the porphyrin levels which were high in long days and low in short days. The gonadotropins are not involved in the control of HG porphyrin content as noted in our experiment in *F. pennanti*.

However, a partial involvement of gonadotropins and steroids on HG function may be suggested for the diurnal squirrel *F. pennanti* as LH injection increased HG weight and testosterone injections decreased HG weight in male squirrels only. More elaborate experiments covering expression of androgen receptor (AR) and estrogen receptor α (ER-α) are needed for a clear understanding. We, therefore, suggest that though gonadotropins and steroids do not have any significant effect on HG functioning of the diurnal squirrel, gonadotropins and steroid hormones influence the pineal and gonadal function negatively. The lack of morphological differences in HGs between male and female diurnal squirrels might be responsible for differential function as noted in *F. pennanti* but a physiological difference at the gender level cannot be ruled out.

Acknowledgments

Authors thank the University Grants Commission, New Delhi, for financial support, and Council of Scientific &Industrial Research (*CSIR*), New Delhi, for the Junior Research Fellowship (JRF) to RV. Equipment grant by Alexander von Humboldt (AvH) to CH is highly appreciated.

References

Berndtson WE, Desjardins C. (1974) Circulating LH and FSH levels and testosterone function in hamsters during light deprivation and subsequent photoperiod stimulation. *Endocrinology* **95**:195-205.

Brainard GG, Vaughan MK, Reiter RJ. (1984) The influence of artificial and natural short photoperiods on male Syrian hamsters: reproductive effects. *Int J Biometeorol*. **28**: 317-325.

Buzzell GR, Menendez-Peleog A, Porkka-Heiskanen T, Pongeri A, Pangeri B, Vaughan MK, Reiter RJ. (1989) Bromocriptine prevents the castration-induced rise in porphyrin concentration in the Harderian glands of the male Syrian hamster, *Mesocrietus auratus*. *J Exp Zool.* **249**:172-176.

Buzzell GR, Hoffman RA, Vaughan MK, Reiter RJ. (1992) Hypophysectomy prevents the castration-induced increase in porphyrin concentrations in the Harderian glands of the male golden hamster: a possible role for prolactin. *J Endocrinol*. **133**: 29-35.

Cardinali DP. (1977) Nuclear receptor estrogen complex in the pineal gland. Modulation by sympathetic nerves. *Neuroendocrinology* **24**:333-346.

Cardinali DP. (1981) Melatonin. A mammalian pineal hormone. *Endocr Rev*. **2**:327-346.

Cardinali DP, Vacas MI. (1978) Feedback control of pineal function by reproductive hormones-a neuroendocrine paradigm. *J Neural Transm Suppl*. **1978**:175-201.

Chieffi G, Baccari CG, Di Matteo L, d'Istria M, Minucci S, Varriale B. (1996) Cell biology of the Harderian gland. *Int Rev Cytol*. **168**:1–80.

Coto-Montes A, Boga JA, Tomás-Zapico C, Rodrí guez-Colunga MJ, Martínez-Fraga J, Tolivia-Cadrecha D, Menéndez G, Hardeland R, Tolivia D. (2001) Physiological oxidative stress model: Syrian hamster Harderian gland-sex differences in antioxidant enzymes. *Free Radic Biol Med*. **30**: 785–792.

Dubey S, Haldar C. (1997) Regulation of Harderian gland porphyrin by pineal gland and melatonin in Indian palm

squirrel *Funambulus pennanti*. *Biogenic Amines* **13**: 101-117.

Haldar C. (1996) Harderian gland function of Indian tropical palm squirrel *F. pennanti*. *Microsc Res Tech.* **34**: 39-47.

Haldar C, Dubey S. (1996) Diurnal variation in circulatory estradiol, testosterone, melatonin and Herderian gland porphyrin concentration in Indian palm squirrel *Funambulus pennanti*. *Indian J Exp Biol*. **34**: 695-697.

Hardeland R, Uria H. (1995) On the role of melatonin in mammalian Harderian gland: does melatonin protect from free radicals generated by protoporphyrin-catalysed photooxidation? *In :* Hardeland R (Ed) *Cellular Rhythms and Indoleamine.* pp145-151. Göttingen: University of Göttingen.

Hoffman RA. (1971) Influence of some endocrine glands, hormones and blinding on the histology and porphyrins of the Harderian glands of golden hamsters. *Am J Anat*. **13:** 463-478.

Marrufo B, Menéndez-Peláe A, Buzzell GR, Gonzalez-Brito A, Reiter RJ. (1989) 5 alpha-dihydrotestosterone administration converts indolamine metabolism and porphyrin content of the female Syrian hamster Harderian gland to the male type. *Proc Soc Exp Biol Med.* **192:** 192-195.

Menéndez-Peláe A. (1990) Melatonin and other indoles in the rodent Harderian glands: regulation and physiological significance. *Advances in Pineal Research*. Vol.4. pp 75-80. London: John Libbey.

Menéndez-Peláe A, Buzzell GR, Gonzelz-Brito A, Reiter RJ. (1990) Androgenic control of N-acetyl transferase activity in the Harderian glands of the Syrian hamster is mediated by 5-dihydrotestosterone. *J Cell Biochem*. **42**:95-100.

Menéndez-Peláe A, Nonaka KO, Hoffman RA, Buzzell GR, Reiter RJ. (1991) Effects of prepubertal manipulation with androgens on the development of sexual differences in the Harderian glands of Syrian hamsters. *Int J Dev Biol*. **35**: 133-138.

Payne AP. (1994) The Harderian gland: a tercentennial review. *J Anat.* **185**: 1-49.

Reiter, RJ, Richardson, B, Hurlbert, EC. (1981) Pineal, retinal and Harderian gland melatonin in a diurnal species, Richardson's ground squirrel (*Spermophilus richardsoni*). *Neurosci Lett*. **22**: 285-288.

Vilchis F, Perez Palacios G. (1989) Steroid hormone receptors and sexual phenotype of the Harderian gland in hamsters. *J Endocrinol*. **121**: 149-156.

Zweens J. (1965) Alterations of the pineal lipid content in the rat under hormonal influences. *Prog Brain Res*. **10**: 540-551.