Proc. Indian natn. Sci. Acad. **B52** No. 3 pp 370-374 (1986)

Effect of Melatonin on the Testis and Thumb Pad of the Skipper Frog, *Rana cyanophlyctis* (Schn.)

H N YAJURVEDI*, A SHANBHAG BHAGYASHRI,** M A HOOLI, and V B NADKARNI
Department of Zoology, Karnatak University, Dharwad 580003

(Received on 12th February 1986; after revision 18th August 1986)

The adult male frogs, *Rana cyanophlyctis* were given intraperitoneal administration of melatonin at 100 μg and 200 μg/frog/day for 15 and 30 days. The melatonin treatment caused a significant decrease in the number of cysts containing the secondary spermatogonia and the primary spermatocytes as well as the nuclear diameter of the Leydig cells. Further, a significant decrease in the diameter of seminiferous tubules was observed following 30 days treatment in frogs treated with melatonin 100 and 200 μg/frog/day, while the height of the epithelial cells of the mucous glands of the thumb pad registered a significant decrease only in frogs treated with 200 μg/frog/day. It is suggested that melatonin at 100 and 200 μg/frog/day induces antigonadal effect in the male *R. cyanophlyctis*.

Key Words : Melation, Testis, Thumb Pad, Rana cyanophlyctis

Introduction

Melatonin exerts both stimulatory and inhibitory effects on gonads in mammals (Turek et al. 1975, Reiter 1981) depending upon the dose, mode, and the time of administration. Studies on the effect of melatonin on the gonadal functions in ectothermic vertebrates are relatively scarce (*see* de Vlaming & Olcese 1981 for references). Though there are a few studies on the effect of melatonin on the testis of amphibians (Juskiewicz & Rakalaska 1963, 1965, de Vlaming et al. 1974, Biswas et al. 1978), these studies do not deal with the effect of melatonin on different population of spermatogenic stages. The present investigation deals with the effect of two different doses (100 and 200μg/frog/day) of melatonin on spermatogenesis, Leydig cells and thumb pad, which is an androgen-dependant secondary sexual character, in *Rana cyanophlyctis*.

Materials and Methods

Adult male frogs, *R. cyanophlyctis* were collected in July, acclimatized to the laboratory conditions for a week and were divided into three groups of 15 specimen each. The frogs were housed in glass aquaria containing tap water, under natural photoperiod and

*Department of Zoology, University of Mysore, Manasagangotri, Mysore
**(Nee) V Bhujli Bhagyashri

were fed on minced thigh muscles of other frogs on alternate day during the course of the experiment. The frogs in the first group received 0·6% saline, 0·2 ml/day/frog, for 15 or 30 days. Melatonin was dissolved in 0·6% saline. All injections were given intraperitoneally at 12·00 hrs each day. Five frogs from each group were autopsied 15 days after the commencement of the experiment and the remaining, after 30 days of treatment. As the half life of melatonin is only for a few minutes, the frogs were sacrificed by decapitation 2 hrs after the last injection.

At autopsy, weights of the body and the testis were recorded. The testis and the thumb pad were fixed in Bouin's fluid for histological and histometric studies. The spermatogenic stages were identified as follows: Stage 0—primary spermatogonia; Stage I—secondary spermatogonia (less than 10 cells in a nest); Stage II—secondary spermatogonia (more than 10 cells in a nest); Stage III—primary spermatocytes; Stage IV—secondary spermatocytes; Stage V—spermatids. The counting of the spermatocysts and the measurement of tubule diameter, Leydig cell nuclear diameter, height of thumb pad epidermis and glandular epithelial height of mucous glands were done as described earlier (Yajurvedi & Hooli 1983). Data from at least five animals were computed for the mean±S.E. and the values in the control and experimental groups were compared using Student's *t* test.

Results

Testis

The testis of all the experimental groups revealed cell nests containing all stages of

Table 1 *Effects of melatonin on the testis of* R. cyanophlyctis

Groups	Testicular wt (mg)/100g Body weight	Diameter (μm)	
		Seminiferous tubule	Leydig cell nucleus
	15 days treatment		
1. Controls	284.59±28.74	274.98±11.9	5.34±0.11
2. 100 μg melatonin/frog/day	232.76±22.98 NS	255.85±10.50 NS	4.38±0.09 $P<0.01$
3. 200 μg melatonin/frog/day	237.52±28.45 NS	233.01±19.17 NS	4.34±0.10 $P<0.01$
	30 days treatment		
4. Controls	301.86±26.55	301.95±10.86	5.60±0.11
5. 100 μg melatonin/frog/day	236.48±17.49 NS	233.10± 4.86 $P<0.01$	4.84±0.24 $P<0.05$
6. 200 μg melatonin/frog/day	239.00±18.14 NS	241.16± 6.09 $P<0.01$	4.72±0.18 $P<0.01$

Values represent mean±S.E. of observations from five specimens
$P<0.05$, $P<0.01$ level of significance with respect of control
NS: Not significant with respect to control

spermatogenesis (Stage 0 to Stage V and sperm bundles).

15 Days Treatment

Following the treatment for 15 days, a significant decrease in the number of cells nests containing secondary spermatogonia (Stage I and II) and primary spermatocytes (Stage III) was observed in the frogs treated with 100μg and 200 μg of melatonin when compared with that of the control (table 2). The nuclei in the Leydig cells in the melatonin treated groups appeared shrunken and hyperchromatic, while such nuclei in the control were round and vesicular. In both the treated groups, there was a significant decrease in Leydig cell nuclear diameter when compared with. that of the control (table 1). There were no significant changes in the number of various spermatogenetic stages and Leydig cells between 100μg and 200μg melatonin treated groups.

30 Days Treatment

Following treatment for 30 days, in both the treated groups, a significant decrease in the diameter of the seminiferous tubules was noted. However, the decrease in the testicular weight was not significant when compared with the control (table 1). In both the treatments, the number of cell nests containing secondary spermatogonia (Stage I and StageII) and primary spermatocytes (Stage III) declined significantly when compared to that of the control (table 2). Leydig cell nuclear diameter also decreased when compared with that of the control (table 1).

Thumb Pad

The mucous gland in the thumb pad of the control frogs were large, provided with papillate epidermis and keratinized spikes. The epithelial cells of the mucous glands contained prominent nuclei. Administration of 200μg melatonin for 15 and 30 days caused a

Table 2 *Effects of melatonin on the spermatogenesis of* R. cyanophlyctis

Groups	Number of cysts belonging to various stages of spermatogenesis/ seminiferous tubule					
	Stage-0	Stage-I	Stage-II	Stage-III	Stage-IV	Stage-V
			15 Days treatment			
1. Controls	4.02±0.28	4.12±0.27	5.22±0.37	5.52±0.37	1.62±0.08	1.29±0.07
2. 100 μg melatonin/frog/-day	3.91±0.12 NS	2.91±0.13 $P<0.05$	3.62±0.18 $P<0.05$	3.91±0.12 $P<0.05$	1.51±0.09 NS	1.12±0.09 NS
3. 200 μg melatonin/frog/-day	3.78±0.09 NS	3.04±0.23 $P<0.05$	3.50±0.45 $P<0.05$	3.84±0.18 $P<0.05$	1.46±0.13 NS	q.08±0.10 NS
			30 Days treatment			
4. Controls	4.05±0.14	3.78±0.12	5.99±0.51	4.99±0.23	1.48±0.11	1.48±0.25
5. 100 μg melatonin/frog/-day	3.69±0.11 NS	2.92±0.13 $P<0.05$	3.98±0.27 $P<0.05$	3.85±0.31 $P<0.05$	1.38±0.14 NS	0.94±0.08 NS
6. 200 μg melatonin/frog/-day	4.09±0.35 NS	3.16±0.17 $P<0.05$	4.96±0.20 $P<0.05$	4.04±0.31 $P<0.05$	1.54±0.08 NS	1.13±0.09 NS

Values represent Mean ± S.E. of observations from five specimens
$P<0.05$; $P<0.01$: level of significance with respect to control
NS: Not significant with respect to control

significant decrease in the height of epithelial cells of mucous glands when compared to those of control (table 3). However, treatment with 100μg melatonin did not alter the epithelial height of mucous glands at 15 or 30 days of treatment when compared to that in control (table 3).

Discussion

In the present study the administration of (100 or 200μg/frog/day) melatonin to *R. cyanophlyctis* did not cause decrease in the relative weight of the testis. However, melatonin at both the dose levels induced a significant decrease in the seminiferous tubule diameter following 30 days treatment. de Vlaming et al. (1974) reported a decrease in testicular weight and seminiferous tubule diameter in the frog *Hyla cinearea* following the treatment with 4 μg melatonin/day/frog for 28 days. A significant decrease in the number of spermatocysts containing secondary spermatogonia and primary spermatocytes/seminiferous tubule suggests an inhibitory action of melatonin on spermatogonia multiplication which subsequently caused reduction in the number of cysts containing primary spermatocytes/seminiferous tubule suggests an inhibitory action of melatonin on spermatogonia multiplication which subsequently caused reduction in the number of cysts containing primary spermatocytes in *R. cyanophlyctis*. However, melatonin did not inhibit hCG-induced stimulation of spermatogenesis in *R. esculenta* (Juskiewicz & Rakalska 1985).

Table 3 *Effects of melatonin on the thumb pad of* R. cyanophlyctis

Groups	Height (μm) of thumb pad	
	Epidermis	Epithelial Cells
15 Days treatment		
1 Controls	67.26±2.58	27.15±0.93
2 100 μg melatonin/ frog/day	63.34±3.12 NS	24.58±0.98 NS
3 200 μg melatonin/ frog/day	60.57±1.44 NS	21.36±0.60 $P<0.05$
30 Days treatment		
4 Controls	72.54±4.38	31.92±0.54
5 100 μg melatonin/ frog/day	71.70±1.68 NS	29.88±0.54 NS
6 200 μg melatonin/ frog/day	62.76±5.52 NS	25.98±0.62 $P<0.05$

Values represent Mean±S.E. of observations from five specimens

$P<0.05$; level of significance with respect to control

NS: Not significant with respect to control

The thumb pads of male anurans which are the androgen dependant secondary sexual character, show correlated changes with the activity of the Leydig cells and are reliable indicators of output of testicular androgens (Lofts 1974). Administration of melatonin reversed the pinealectomy induced increase in the steroidogenic activity in the testis of *Bufo melanostictus* (Biswas et al. 1978) as indicated by the changes in the nuclear morphology of the Leydig cells. The melatonin induced regression of the Leydig cells accompanied by the regression of epithelial cells of mucous gland of the thumb pad in *R. cyanophlyctis*, as revealed in the present study is suggestive of the impairment of steroidogenic activity of the Leydig cells.

It is suggested that there is a functional relationship between pineal complex and pituitary gonadal axis (de Vlaming & Olcese 1981). It is possible that melatonin might effect the gonadotropin(s) secretion and thereby induce changes in testicular activity, since, in amphibians the proliferation of the spermatogonia and stimulation of the Leydig cells are gonadotropin dependant (Lofts 1974). The effect of melatonin on gonadotropin(s) secretion is evidenced by the inhibitory effect

on the secondary spermatogonial population as well as the Leydig cells in *R. cyanophlyctis*. The decrease in cysts containing primary spermatocytes might be the result of occurrence of less number of cysts containing secondary spermatogonia. Similar changes have also been observed in the testis by the inhibition of hypophyseal gonadotropin release following sex steroid treatment in *R. cyanophlyctis* (Yajurvedi 1984). It, therefore appears probable that action of melatonin may be mediated through hypophyses in *R. cyanophlyctis*. However, further experimentation is required to define precisely how melatonin inhibits gonadal function in frogs.

Acknowledgements

One of us (HNY) is grateful to the CSIR, New Delhi for a Senior Research Fellowship. We are thankful to the Karnatak University for laboratory facilities.

References

Biswas N M, Chakraborty J, Chenda S and Sanyal S 1978 A basic experimental approach in perspective of pineal and melatonin involvement in photoperiod-induced alteration of spermatogenesis in toad (*Bufo melanostictus*); *Endocrinologie* **71** 143–148

De Vlaming V L, Sage M and Charlton C B 1974 The effect of melatonin treatment on gonosomatic index in the teleost, *Fundulus similis* and the tree frog, *Hyla cinerea; Gen. Comp. Endocrinol.* **22** 433–438

——and Olcese J 1981 The pineal and reproduction in fish, amphibians, and reptiles; in *The Pineal Gland, Vol. II: Reproductive Effects* pp 1–29 ed. R J Reiter (Florida : CRC Press Inc.)

Juskiewicz T and Rakalska Z 1963 Anti-oestrogenic effects of bovine pineal glands; *Nature* (London) **200** 1329–1330

—— and —— 1965 Lack of the effect of melatonin on the frog spermatogenic reaction; *J. Pharm. Pharmacol.* **17** 189

Lofts B 1974 Reproduction in; *Physiology of Amohibia; Vol. II* pp 197–218 ed. B Lofts (New York; London; Academic Press)

Reiter R J 1981 *The Pineal Gland; Vol. II Reproductive Effects* (Florida; CRC Press Inc.)

Turek F W, Desjardins C and Menaker M 1975 Melatonin antigonadal and progonadal effects in male golden hamsters; *Science* **190** 280–282

Yajurvedi H N 1984 Some studies on the Interrenal gland and the testis in relation to the pituitary gland of the Indian skipper frog *Rana cyanophlyctis* (Schn.); PhD Thesis, Karnatak Uni. Dharwad India

—— and Hooli M A 1983 Effect of progesterone on the testis, thumb pad and pars distalis of the skipper frog *Rana cyanophlyctis* (Schn.); *Proc. Indian natn. Sci. Acad.* **B49** 620–627