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Effect of gonadotropins and alpha 2u-globulin on testicular steroidogenesis and spermatogenesis in melatonin-treated rats

R. Biswas[1], N. M. Biswas[2], H. Mandal*, N. M. Biswas[3]

[1]Post doctorate fellow, Department of Oncology, National Cancer Institute, Bethesda, Maryland, USA

[2]Associate Professor, Department of Medicine, NRS Medical College, Calcutta-14, India; [3]Professor in Physiology, College of Medical Sciences, Bharatpur, Nepal; *Deceased before this communication.

Abstract

Administration of melatonin (400μg/100g bd.wt.) for 14 days caused a fall in weights of the testes and accessory sex organs and testicular 17β-hydroxysteroid dehydrogenase (17β-HSD) but rise in 3β-hydroxysteroid dehydrogenase (3β-HSD) activity, decreased spermatogenesis, serum level of gonadotropins, testosterone and alpha 2u-globulin, The animals treated with melatonin when received gonadotropins or alpha 2u-globulin for the last seven days reversed the weight of testis and accessory sex organs, 3β-HSD, 17β-HSD activities, serum level of gonadotropins, testosterone and alpha 2u-globulin when compared with melatonin-treated rats. It is concluded that alpha 2u-globulin prevents testicular degeneration in melatonin-treated rats by stimulating the synthesis of gonadotropins.

Key words: Alpha 2u-globulin, gonadotropins, male gonad, melatonin.

Introduction

A sex-dependent urinary protein, alpha 2u-globulin is synthesized in the liver and due to its small molecular size it is cleared by the kidneys in the adult male rats.[1,2] Sexually mature male rats show diurnal rhythm of alpha 2u-globulin synthesis.[3] Similar diurnal rhythm also occurs in melatonin synthesis from the pineal gland in rats.[4] On the other hand melatonin treatment causes a substantial decrease in FSH together with testicular atrophy.[5] While perfusion of the third cerebroventricle with melatonin lowers the plasma concentration of both FSH and LH in the adult rats.[6] Melatonin treatment also decreases serum level of gonadotropins and alpha 2u-globulin along with the inhibition of testicular activity in the adult rats.[7]

Correspondence: N. M. Biswas
E-mail: drnmbiswas@gmail.com

The purpose of the present investigation was to determine whether external administration of FSH, LH or alpha 2u-globulin can prevent testicular degeneration in melatonin-treated rats.

Materials and methods

Animal-Adult male rats of Sprague Dawley (*Rattus norvegcus*) strain weighing 150-200g were used in this experiment. A standard laboratory chow and water were available ad-libitum.

Preparation of alpha 2u-globulin

Alpha 2u globulin was isolated from male rat urine according to the method of Roy, Neuhaus and

Harmison.[8] The antiserum of alpha 2u-globulin was raised in Dutch belted rabbits by injecting an emulsion of equal volume of alpha 2u-globulin and freund's complete adjuvant. Immunoassay of serum alpha 2u-globulin was carried out in calibrated plastic immunodiffusion plates as described previously[9]

Melatonin, alpha 2u-globulin, FSH and LH treatment

Forty rats were divided into five equal groups. Thirty two rats received subcutaneous injection of melatonin daily between 19.00 and 20.00hr for 14 days.

Melatonin received from Sigma Chemical Company, St. Louis, MD.USA, dissolved in vehicle (ethanol and water, 10:90, V/V) and injected 400μg/100mg bd.wt./day. The melatonin treated rats were divided into four groups, one of which received no further treatment (Group II). From day eight of melatonin treatment, Group III animals treated with FSH and Group IV animals with LH. Both the hormones dissolved in distilled water and injected s.c.25μg/100g.bd.wt./day every morning for the last seven days. The animals of Group IV received alpha 2u-globulin, dissolved in distilled water and injected s.c. at a dose of 1.5mg/rats/day for the last seven days of melatonin treatment. Remaining eight animals received vehicle only (Group I). Blood was obtained from all the animals after 24hrs of last injection and serum was collected by centrifugation. All the animals were killed, the testes and secondary sex organs weighed.

Assay of testicular 3β-HSD and 17β-HSD activities and testicular histology

One testis of each animal was used to measure the activity of 3β-HSD and 17β-HSD.[10, 11] The remaining testis from each animal was fixed in Bouin's fixative and embedded in paraffin wax. Sections (5μm thick) stained with periodic acid -schiff-haematoxylin. Quantitative analysis of the seminiferous epithelium was carried out by counting the number of germ cell nuclei per cross section of the seminiferous tubule at the stage VII of the cycle of seminiferous epithelium. Germ cell nuclei were counted in 25 round tubular cross-sections at the VII of the cycle. All the nuclear counts of the germ cells were corrected for differences in nuclear diameter[12] and for the tubular shrinkage by a sertoli cell correction fector.[13]

Hormone Assay - Serum FSH and LH were measured by radioimmunoassay according to the method of Moudgal and Madhwa Raj[14] using reagents supplied by Rat Pituitary Distribution Program, NIAMDD, Maryland, USA. Serum samples were assayed in duplicate and gonadotropins were expressed as μg/litre serum. The radioimmunoassay of testosterone was carried out as described by Auletta et al.[15] The antiserum to testosterone was purchased from the Endocrine Science, USA, and had a 44% cross reactivity with dehydrotestosterone.The testosterone values are sum of testosterone and dehydrotestosterone, since chromatographic purification of the samples was not performed.

Data were analyzed by using Student's t-test.

Results

Adult rats were given melatonin for 14 days and killed on day 15 showed decreased testicular and accessory sex organ weights in comparison with vehicle - treated controls (Table-1). A reduced 17β-HSD activity of the testis and serum alpha 2u-globulin but an elevation of 3β-HSD activity of the testis was observed in melatonin treated rats. Serum levels of FSH, LH and testosterone

were reduced after melatonin treatment when compared with control animals. Histological examination of the seminiferous tubules at stage VII of the cycle revealed that melatonin treatment caused a marked reduction of type aspermatogonia, preleptotene spermatocytes and spermatids at this stage. The reduced weights of testes and sex-accessories caused by melatonin treatment were increased after FSH, LH or alpha 2u-globulin administration. The changes of the activities of steroidogenic enzymes and serum level of alpha 2u-globulin due to melatonin treatment have been showed to improve towards the control values after administration of FSH, LH and alpha 2u-globulin (Table-2). Serum levels of FSH, LH and testosterone were also increased after administration of FSH, LH or alpha 2u-globulin in relation in treated rats when compared with the rats treated with melatonin alone (Table-3). Testicular spermatogenic arrest due to melatonin treatment was reversed after administration of FSH, LH or alpha 2u-globulin (Table 4 & 5).

Table 1: Weights of testis and accessory sex organs after administration of gonadotrophins and alpha 2u-globulin in Melatonin treated rats.

Treatment	Testis Weight (mg/100g bd.wt.)	Seminal Vesicle weight (mg/100g bd.wt.)	Ventral Prostate (mg/100g bd.wt.)
Group I: Control animals	1629.34±63.48	435.45±37.25	187.25±27.31
Group II Melatonin	1271.67±46.32	245.73±41.12	73.46±26.57
Group III Melatonin + FSH	1567.27±51.14	401.19±42.25	168.29±24.02
Group IV Melatonin + LH	1551.38±42.42	398.16±40.25	171.18±24.25
Group V Melatonin + alpha 2u-globulin	1552.18±47.12	412.39±32.38	176.47±22.19

Values of Groups I, III, IV & V are significantly different from the Group II ($P<0.05$).

*Each value represents mean ± SE of 8 animals in each group.

Table -2: Testicular 3β-HSD and 17β-HSD activities and serum level of alpha 2u-globulin after administration of gonadotropins and alpha 2u-globulin in melatonin treated rats.

Treatment	3β-HSD activity unit/mg of tissue/hr	17β-HSD activity unit/mg of tissue/hr	Alpha 2u-globulin mg/100 ml
Group I: Control animals	23.42±0.48	25.87±0.58	2.48±0.18
Group II Melatonin	26.87±0.53	21.98±0.55	1.02±0.17
Group III Melatonin + F FSH	23.21±0.48	24.92±0.49	2.22±0.21
Group IV Melatonin + LH	23.14±0.51	24.79±0.52	2.18±0.16
Group V Melatonin + alpha 2u-globulin	23.38±0.61	24.68±0.62	2.64±0.21

Each value represents mean ±SE of 8 animals in each group. Values of the Groups I, III, IV & V are significantly different from the Group II ($P<0.05$).

Table -3: Serum levels of FSH, LH and testosterone after administration of FSH, LH and alpha 2u-globulin in melatonin treated rats.

Treatment	FSH (μg/L)	LH (μg/L)	Testosterone (μg/L)
Group I: Control animals	181.82±3.16	38.18±1.41	3.22±0.04
Group II: Melatonin	143.37±2.05	18.99±1.85	2.17±0.03
Group III: Melatonin + FSH	179.88±2.73	36.41±2.05	3.05±0.05
Group IV: Melatonin + LH	163.18±3.07	37.92±1.68	2.98±0.04
Group V: Melatonin + alpha 2u-globulin	174.28 ±2.98	35.83±1.67	3.09±0.06

Each value represents mean ±SE of 8 animals in each group. Values of the Groups I, III, IV & V are significantly different from the Group II ($P<0.05$).

Table -4: Quantitative analysis of spermatogenesis at stage VII in the seminiferous tubules after administration of gonadotropins and alpha 2u-globulin in melatonin-treated rats.

Treatment	Preleptotene Spermatocytes	Type A Spermatogonia	Pachytene Spermatocytes	Spermatid
Group I: Control animals	15.83±0.27	0.56±0.04	16.16±0.21*	56.86±1.22
Group II: Melatonin	13.61±0.33	0.39±0.02	15.92±0.26*	41.69±0.68
Group III: Melatonin + FSH	15.64±0.27	0.51±0.04	15.98±0.24*	49.72±1.16
Group IV: Melatonin LH	15.62±0.28	0.49±0.03	16.01±0.25*	50.19±0.98
Group V: Melatonin alpha 2u-globulin	15.78±0.26	0.53 ±0.04	16.07±0.18*	52.61±1.06

*Represent statistically nonsignificant. Other Values of Group I, III, IV, V are significantly different from the Group II ($P<0.05$). Each value represents mean ± SE of 8 animals in each group.

Table -5: Pachytene spermatocyte: Spermatid ratio of the seminiferous tubule at stage VII and percentage of degeneration of step 7 spermatid after administration of gonadotropins and alpha 2u-globulin in melatonin-treated rats.

Treatment	Pachytene Spermatocyte: Spermatid ratio	% of spermatid degeneration
Group I: Control animals	1:3.52	12.00
Group II: Melatonin	1:2.62	34.50
Group III: Melatonin + FSH	1:3.11	22.25
Group IV: Melatonin + LH	1:3.13	21.75
Group V: Melatonin + alpha 2u-globulin	1:3.27	18.25

Discussion

These results show that administration of gonadotropins or alpha 2u-globulin in melatonin-treated rats increased serum level of testosterone and prevented the arrest of spermatogenesis. Melatonin treatment of rats stimulated testicular 3β-HSD and inhibited 17β-HSD in a manner comparable to estrogen treated rats.[16] The reduction of 17β-HSD activity after melatonin treatment is possibly due to reduced gonadotropin secretion. On the other land 17β-HSD is one of the key enzymes of androgen synthesis. So the decreased level of gonadotropin dependent serum testosterone after melatonin treatment is, therefore, a reflection of reduced secretion of pituitary gonadotropins. The stimulation of 3β-HSD activity may increase the synthesis of progesterone as suggested in studies using slices of testis taken from estrogen -treated rats.[17]The quantitative analysis of seminiferous tubules at stage VII of the cycle revealed that melatonin treatment reduced the number of spermatogonia, preleptotene spermatocytes and spermatids. Theoretically the pachytene spermatocytes: Spermatids ratio should be 1:4[13] in our control ratio is 1:3.52 i.e. 12% of spermatid degeneration. This ratio became 1:2.62 in the melatonin-treated rats indicating that during the process of conversion of spermatocyte to spermatid 34.50% of the cells degenerated. Since FSH inhibits degeneration of spermatogonia[18] and conversion of pachytene spermatocyte to spermatid which requires LH-induced testosterone[19] in the present experiment spermatogenic arrest and degeneration of spermatocytes have been prevented in melatonin treated rats after administration of FSH, LH or alpha 2u-globulin. So the stimulation of spermatogenesis and testicular steroidogenesis in melatonin-treated rats is possibly an indirect effect of alpha 2u-globulin by inducing pituitary gonadotropins.[16]

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