Effect of continuous light on spermatogenesis and testicular steroidogenesis in rats: Possible involvement of alpha 2u-globulin

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ABSTRACT

Male rats exposed to continous light for 70days showed an increased weights of testis, accessory sex organs, serum levels of FSH, LH, testosterone, testicular 17β-hydroxysteroid dehydrogenase (17β-HSD) activity and alpha 2u-globulin, while 3β-hydroxysteroid dehydrogenase activity showed no significant changes. Prolonged light exposure also stimulated sepermatogenesis in rats. These results suggest that alpha 2u-globulin possibly stimutates the male gonad by inducing pituitary gonadotropins in continuous light-exposed rats.

Keywords: Constant light, alpha 2u- globulin, testis.

INTRODUCTION

Photostimulation in the immature golden hamster increases testicular activity and testosterone secretion.[1] But in adult marmoset (Callithrix Jacehus), continuos light exposure for 60 days has no effect on the rhythmicity of plasma testosterone and spermatogenesis.[2] On the other hand long term exposure to continuous light inhibits gonadal growth in European male seabass.[3] Similarly constant light exposure in adult rats decreases LH secretion while FSH level is significantly higher.[4] On the other hand constant illumination in toad (Bufomelanostictus) suppresses spermatogenesis along with increase in Leydig cell activity, possibly due to stimulation of LH secretion.[5] Furthermore, photostimulation inhibits the synthesis of melatonin while synthesis of an urinary protein, alpha 2u-globulin increases in rats.[6] Since alpha 2u-globulin plays an important role in male gonadal function in rats,[7] the present investigation has been undertaken to determine the effect of continuous light exposure on gonads and the role of alpha 2u-globulin in the gonads of photostimulated male rats.

MATERIALS AND METHODS

Experiments were carried out on 50-60g adolescent male rats of the wistar strain with a standard laboratory chow and water *ad libitum.* Twenty rats were divided equally into two groups. Groups 1, control animals, kept under natural light and dark exposure (L:D-14:10) for 70 days. Group II animals kept under constant light exposure for 70 days. All animals were sacrificed after 70 days. Blood was obtained from all animals and serum was collected by centrifugation. The accessory sex organs were dissected out and weighed. One testis from each animal was fixed in Bouin's fluid for histological studies while other testis was used for assay of 3β-hydroxy-steroid dehydrogenase (3β-HSD) and 17β-hydroxy-steroid dehydrogenase (17β-HSD).[8,9] Alpha 2u-globulin was prepared from male rat urine.[10] The antiserum to alpha 2u-globulin was raised in Dutch belted rabbits by injecting an emulsion of equal volume of alpha 2u-globulin and Freund's complete adjuvent. Immunoassay of serum alpha 2u-globulin was carried out in calibrated plastic immunodiffusion plates as described previously.[10] The section of testis was stained with periodic acid Schiff(PAS)- hematoxylin. The quantitative analysis of seminiferous epithelium was carried out on the basis of relative number of germ cell nuclei per cross-section of seminiferous tubules at the stage VII of the cycle of the seminiferous epithelium. Germ cell nuclei were counted in 25 round tubular cross-section at the stage VII of the cycle. All the nuclear counts of the germ cells were corrected for differences in nuclear diameter by the formula of the Abercrombie,[11] for tubular shrinkage by a sertoli cell correction factors.[12]

HORMONE ASSAY

Serum FSH and LH were measured by radioimmunoassay according to the method of Moudgal and Madhwa Raj.[13] Serum samples were, assayed in duplicate and the amount of gonadotropins were expressed as μg/litre serum. The radioimmunoassay of testosterone was carried out as described by Auletta *et al*.[14] All samples were run in duplicate in a single assay to avoid interassay variation. The interassay coefficient of variation was 6.5%.

RESULTS

Male albino rats kept under constant light for 70 days, showed an increased weights of testis, seminal vesicle and ventral prostate in comparison to the control animals (Table-1). An increased 17β-HSD activity and an insignificant change of 3β-HSD activity were also observed in the testes of rats exposed to continuous light (Table-2). Serum levels of LH, FSH, testosterone and alpha 2u-globulin were increased in the rat exposed to continuous light in comparison to control animals

(Table-3). Quantitative study of the germcell at the stage VII revealed that continuous photostimulation resulted in a significant increase in the number of type A spermatogonia, preleptotene spermatocytes and spermatids in comparison to control animals (Table-4), prolonged light exposure also prevented normal degeneration of spsmatids to a considerable extent (from 19.25% to 8.5%) (Table-5).

DISCUSSION

Light and dark cycle in circadian rhythm plays a significant role in endocrine and reproductive function.[15-17] The present experimental results show that constant exposure to light for 70 days stimulated the male gonadal activity in adolescent rats. Photostimulation of golden hamsters (14:10 hr light:dark) from birth to early puberty also stimulated testicular testosterone secretion.[1] Similar increase in serum level of testosterone observed in the present experiment due to increase in 17β-HSD activity after continuous light exposure. Since gonadotropins are responsible for stimulating the enzymes involved in steroid hormone synthesis,[18] an increasesed serum levels of FSH and LH possibly elevated testosterone

Table-1: Weights of testes and accessory sex organs in the rats kept under constant light exposure for 70 days. Each value represents mean ± SE of 10 animals in each group

Treatment	Testis Weight (mg/100g body weight)	Seminal Vesicle weight (mg/100g body/weight)	Ventral prostate (mg/100g body weight)
1. Control animals light and dark (L:D::14:10)	1405.18±31.04	327.37±22.01	161.06±16.04
2. Constant light exposure	1498.7±322.4	393.84±21.62	209.93±16.11

P<0.05 compared with controls (analysis of variance and Duncan's test)

Table-2: Testicular 3β-HSD activities and serum level of alpha 2u-globulin in the rats kept under constant light exposure for 70 days

Treatment	3β-HSD (Unit/mg tissue per h)	17β-HSD (Unit/mg tissue per h)	Serum alpha 2u globulin mg/100ml
1. Control animals light and dark (L:D::14:10)	23.16 ± 0.38	25.57 ±0.28	2.41 ± 0.16
2. Constant light exposure	22.58±0.31	26.18±0.32*	2.98±0.18*

Each value represent mean ±SE of 10 animals in each group.

*P<0.05 Compared with controls (analysis of varience and Duncan's test)

Table-3: Serum levels of FSH, LH and testosterone in the rats kept under constant light exposure for 70 days

Treatment	FSH mg/L	LH mg/L	Testosterone mg/L
1.Control animals light and dark (L:D::14:10)	189.22 ±2.36	35.43±1.78	3.36± 0.06
2.Constant light exposure	208.20±3.72*	45.04±1.02*	3.78±0.08*

Each value represent mean ±SE of 10 animals in each group

*P<0.05 Compared with controls (analysis of varience and Duncan's test)

Table-4: Changes in relative number of germ cell nuclei per cross section of seminiferous tubules at stage vii of spermatogenesis in rats kept under constant light for 70 days

Treatment	Type A Spermatogonia	Preleptotene Spermatocytes	Pachytene spermatocytes	Spermatids
1.Control animals light and dark (L:D::14:10)	0.51 ± 0.03	14.04±0.22	16.13± 0.37	52.14±1.05
2.Constant light exposure	0.64±0.04*	14.98±0.21*	16.17±0.32	59.17±0.81*

*P<0.05 Compared with controls (analysis of varience and Duncan's test)

Each value represent mean ± SE of 10 animals in each group

Table 5: Pachytene spermatocyte: Spermatid ratio of seminiferous tubules at stage VII and percentage of degeneration of step 7 spsmatid in the rats kept under constant light for 70 days

Treatment	Pachytene spermatocytes Spermatids ratio	% of spermatids degeneration
1.Control animals light and dark (L:D::14:10)	1:3.23	19.25
2.Constant light exposure	1:3.66	8.25

by stimulating its synthesis in photostimulated rats. An increased serum level of alpha 2u-globulin in light exposed rats is possibly due to an increased synthesis of testosterone as alpha 2u-globulin synthesis is stimulated by androgen.[19]

The quantitative analysis of seminiferous tubules at stageVII of spermatogenesis reveled that continuous illumination increased the number of spermatogonia, preleptotene sprmatocytes and step VII spermatids. Theoritically, the pachytene spermatocytes: spermatids ratio should be I:4.[12] and was found to be 1:3.23 in our control rats. This ratio increased to 1:3.66 in light-exposed rats indicating that during the conversion of spermatocytes to spermatids only 8.50% of the cells were degenerated while in control animals the degeneration is 19.23%. Increase in number of preleptotene spermatocyte in light-exposed rats is possibly due to formation of more number of spermatogonia. Since FSH prevents the degeneration of spermatogonia[20] and testosterone plays an important role in the conversion of spermatocytes to spermatids,[21]in the present experiment continuous light may have increased the number of germ cells by stimulating both gonadotropins and testosterone. Photostimulation also increased alpha 2u-globulin. The mechanism of gonadal stimulation by endogenous alpha 2u-globulin in light exposed rats can not be determined from the present study. Previous observation indicates that exogenous administration of alpha 2u-globulin prevents testicular degeneration by inducing gonadotropins and testosterone synthesis in melatonin-treated rats.[22] So the stimulation of gonadal activity in male rats exposed to prolonged photostimulation is possibly due to increased secretion of gonadotropins through the induction of alpha 2u-globulin synthesis.

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