

Role of light in the mediation of acute effects of a single afternoon melatonin injection on steroidogenic activity of testis in the rat

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Young adult male rats, maintained either in an LD 12 : 12 or in continuous illumination (LL) for one week, were given a single injection of 25 µg melatonin/100 g body wt or ethanolic-saline (control) at 17:00 h. Animals from each group were sacrificed at 11:00 h on the following day. The activity of two important steroidogenic enzymes, 17 β -hydroxysteroid dehydrogenase (17 β -HSD) and Δ^5 -3 β -hydroxysteroid dehydrogenase (Δ^5 -3 β -HSD), and serum concentrations of testosterone, were measured following highly specific and sensitive spectrophotometric techniques and RIA, respectively. A significant decrease in the activity of both the steroidogenic enzymes was noted in the testes of melatonin-treated rats maintained under normal light-dark schedules, but this response was found to be lacking in the LL rats. However, no significant changes in the level of serum testosterone were noted in either group of melatonin-treated rats from the values in respective groups of ethanolic saline-administered LD and LL rats. Exposure of ethanolic saline-injected rats to continuous light also did not cause any change in the steroidogenic activity of the testis from those in LD rats. The study indicates that continuous light as such does not affect the endocrine function of testis but abolishes suppressive effects of melatonin on the steroidogenic activity of the testis in rat.

1. Introduction

The pineal gland in higher vertebrates is believed to act as a photo-neuroendocrine transducer and transmits the photoperiodic information to various physiological processes in general and gonadal growth and activity in particular (Pevet *et al* 1999). Most of the studies have shown that exogenous administration of pineal hormone melatonin to various groups of animals lead to suppression of gonadal activity, though the effects are dependent on the dose (Turek and Pappas 1980; Petterborg 1986), duration (Amador *et al* 1988; Olivares *et al* 1989) and time (Hong and Stetson 1987) of administration of hormone, as well as the age of the animal (Donham *et al* 1989), season of treatment (Maitra and Dey 1992) and the photoperiod to which the animals are exposed (Maitra and Dey 1996). Generally melatonin-induced gonadal atrophy is known to be a gradual process and requires about 4 to 12 weeks to

complete the effect (Reiter 1991a). It is presumed that melatonin acts to produce subtle but progressive alterations in the neuroendocrine-reproductive axis (Reiter 1981). However, information is also available to show that a single afternoon melatonin injection has immediate effects on several adenohipophyseal hormones important in the regulation of reproduction in the Syrian hamster (Petterborg *et al* 1984). Several studies have shown that endogenous synthesis of melatonin in almost all the studied animals is acutely light sensitive and the nocturnal melatonin level is diminished by the exposure of light pulse during night (Illnerova *et al* 1983; Vakkuri *et al* 1985; Maitra *et al* 1986). However, it remains to be known whether exposure of light following acute administration of melatonin influences gonadal activity in any animal. Thus an attempt has been made in the present investigation to explore the importance of light in the mediation of reproductive effects of a single afternoon melatonin injection in rats.

Keywords. Illumination; melatonin; pineal; rat testis; steroidogenesis

2. Materials and methods

Twenty young adult male white rats weighing between 125–135 g were purchased from a local animal dealer. Prior to any experimentation, they were maintained for a week under laboratory conditions including natural light supplemented with artificial light from fluorescent source between 8:00 h to 18:00 h for a week with food and water *ad libitum*. Subsequently, the rats were evenly divided into two groups and were housed in separate rooms with different lighting conditions; one with normal light-dark condition and another with continuous light (LL). Following a 7-day period for different light exposure, half of the total rats in each group received a single subcutaneous injection of melatonin (Sigma; 25 µg in 0.1 ml ethanolic-saline) while the other group was given only the ethanolic-saline. The injections were given at 17:00 h, i.e. 1 h prior to onset of darkness in LD group of animals. On the following day, body weight of individual rats was recorded and all the rats, irrespective of their experimental conditions, were killed between 11:00 and 12:00 h, i.e. after about 18 to 19 h of the injections. Following decapitation, trunk blood samples were collected in heparinized tubes, centrifuged, and the plasma thus obtained was frozen and stored until assayed for testosterone. The paired testes were collected, blotted for soaking extraneous water and tissue fluids, and weighed in a balance with a sensitivity of ± 0.01 mg. The left testis of each rat was fixed in aqueous Bouin's solution, dehydrated in graded alcohol, and embedded in paraffin to obtain 5 µm thick sections following routine tissue preparation technique. The right testis of each rat was homogenized in a medium (5 ml) containing 20% glycerol, 5 mM potassium sulphate and 1 mM EDTA. The preparation was centrifuged at 10,000 g at 4°C for 30 min, the supernatant was used for spectrophotometric assay of 17 β -hydroxysteroid dehydrogenase (17 β -HSD) and Δ^5 -3 β -hydroxysteroid dehydrogenase (Δ^5 -3 β -HSD) using testosterone and dehydroandrosterone as the substrates respectively (Jarabak 1969). The activity of each enzyme was expressed as absorbance/mg protein/min. The protein content of each testis was measured following the method of Lowry *et al* (1951).

Plasma levels of testosterone were measured by the radioimmunoassay (RIA) using a commercial testosterone I¹²⁵ RIA kit, Cat no. 07-289102, procured from M/s ICN Biomedicals, Diagnostics Division, CA, USA. We slightly modified the method in our laboratory.

One way analysis of variance (ANOVA) was followed by Student's *t* test (Zar 1974) for statistical analysis of quantitative data on each parameter of study in different groups of experimental rats. The criterion for significance was taken as ≤ 0.05 .

3. Results

Mean values of body weight and paired testicular weight did not show any significant difference between the different groups of ethanolic-saline administered and melatonin treated rats under any light-dark condition (table 1). The histomorphological study of testes also could not detect any response to the experimental conditions used in this investigation. However, a significant decrease in the activity of two important steroidogenic enzymes, i.e. 17 β -HSD and Δ^5 -3 β -HSD was noted in the testes of melatonin treated rats maintained under normal light-dark schedules, but this response was found to be lacking in the LL rats (table 1). On the other hand, no significant changes in the level of serum testosterone were noted in either group of melatonin treated rats from the values in ethanolic-saline administered LD and LL rats respectively (table 1). Exposure of ethanolic-saline injected rats to continuous light also did not cause any subtle change in the steroidogenic activity of the testis and the serum concentrations of testosterone from those in LD rats (table 1).

4. Discussion

Melatonin synthesis within the body in all the animals investigated so far is highly rhythmic with a peak in the mid-night and nadir at mid-day (Reiter 1991b). The duration of nocturnal peak in melatonin is dependent on the duration of darkness (Goldman *et al* 1984). One of the most characteristic features of melatonin biosynthesis is

Table 1. Mean values (\pm SE) of body weight, paired testicular weight, activity of two important steroidogenic enzymes, i.e. 17 β -HSD and Δ^5 -3 β -HSD in the testis, and plasma concentrations of testosterone in ethanolic-saline or melatonin administered rats maintained under 12L : 12D light dark (LD) schedule or under continuous illuminations (LL).

Animal group	Body wt. (g)	Paired testicular wt (g)	Testicular 17 β -HSD activity (absorbance/min/mg protein)	Testicular Δ^5 -3 β -HSD activity (absorbance/min/mg protein)	Plasma level of testosterone (ng/ml)
Control (LD)	132.00 \pm 5.62	1.75 \pm 0.05	0.0193 \pm 0.001	0.0230 \pm 0.001	0.674 \pm 0.247
Melatonin (LD)	131.00 \pm 5.12	1.86 \pm 0.51	0.0155 \pm 0.001*	0.0140 \pm 0.001*	0.676 \pm 0.226
Control (LL)	133.00 \pm 4.98	1.66 \pm 0.08	0.0180 \pm 0.002	0.0205 \pm 0.001	0.756 \pm 0.234
Melatonin (LL)	133.00 \pm 1.22	1.62 \pm 0.07	0.0140 \pm 0.001	0.0194 \pm 0.002	0.730 \pm 0.245

**P* < 0.01.

that the mechanism is highly light sensitive (Maitra *et al* 1986). It has been well documented that continuous light (LL) exposure either dampens or inhibits pineal melatonin rhythms (Binkley 1986). The study also led to suggest that exposure of any animal to LL results in "functional pinealectomy" (Carter *et al* 1982). Wurtman *et al* (1963) first observed that prolonged exposure to constant light in rats of either sex accelerates the gonadal growth and activity. They also reported constant darkness induced involution of the gonads. In hamsters, the effect of LL on the gonadal activity depends upon species. In Turkish hamsters, continuous illumination causes testicular regression (Carter *et al* 1982; Hong *et al* 1986), whereas in golden and Djungarian hamsters LL appears to have no different effect on testicular functions than any other long day (Elliot 1976; Goldman *et al* 1982). Species specific response of pineal to continuous illumination is also reported in literature (Vollrath and Maitra 1986).

In the present study, the exposure of rats to LL for a week did not cause any subtle change in any of the reproductive parameters from those maintained in LD schedule. It is thus clear that the adopted experimental schedule may have dampened the melatonin rhythm in pineals (Binkley 1986), but that may not be sufficient to alter the functional status of pineal-gonadal axis in rats (Elliot 1976). The gonadal responsiveness to LL may vary depending upon the age of the animal (Oishi 1978), duration of exposure (Kumar and Tewary 1983), and even the intensity of light (Sikdar and Kar 1994). However, the role of light, and hence of darkness, in the mediation of effect of exogenous melatonin is evident from the results of the present study showing a suppressive effect of administered melatonin on the steroidogenic activity of testis in LD but not in LL rats. Since exposure of animals to LL is reported to suppress endogenous synthesis of melatonin, there is a possibility that the anti-gonadal response to administered dose of melatonin under LD conditions (Petterborg *et al* 1984; this study) may not be due to its influence alone, but rather a result of interactions between the endogenous and exogenous hormones (Skene *et al* 1987).

The endocrine mechanism of an antigonadal effect of melatonin is as yet a topic of conjecture. Available information suggests that melatonin may act through the hypothalamic receptors to inhibit luteinizing hormone (LH)-releasing hormone secretion (Reiter *et al* 1981), or directly inhibit the secretion of pituitary LH (Yamashita *et al* 1977; Petterborg *et al* 1984), or directly act on the testis to suppress the enzymatic activity for steroidogenesis (Ellis 1972). It is notable that in the present study the activity of two important steroidogenic enzymes, i.e. 17 β -HSD and Δ^5 -3 β -HSD was measured using testosterone and dehydroepiandrosterone as the substrate respectively, and a suppression of activity of either enzymes occurred in melatonin administered LD rats. The results suggest an

inhibitory role of melatonin on conversion of both dehydroepiandrosterone to androstenedione and androstenedione to testosterone (Rommerts 1990), though the circulatory level of testosterone in either group of animals remained almost unaltered irrespective of the treatment. This may possibly be due to the fact that the level of testosterone in blood samples of melatonin administered LD rats was largely contributed by the testes before the onset of treatment, as the half-life of androgens is known to be more than 24 h (Harris *et al* 1977). In other words, it may be surmised that under LD conditions an antiandrogenic influence of melatonin is due to suppression at the biosynthetic pathway level of testosterone but not because of an altered rate of clearance or utilization of testosterone at tissue level (Elliot 1976). However, additional supportive evidence should be available in support to the alleged effect of melatonin. Since spermatogenesis in rats and other mammals is under the control of testosterone (Weinbauer and Nieschlag 1990), an absence of any gametogenic response to melatonin administration in either group of rats is explainable.

Generally reproductive effects of pineal or melatonin are studied following prolonged treatment, but it is equally important to demonstrate the mechanism involved in such study from the beginning. The experimental schedules, including the dose of melatonin, used in this study was the same as followed with an earlier study on Syrian hamster (Petterborg *et al* 1984), where a single afternoon melatonin injection resulted in a significant reduction in the levels of pineal melatonin and pituitary LH after 18 h of the treatment. The present study shows for the first time that within the given time the steroidogenic activity of similarly treated rats is also suppressed, only when the animals were allowed to experience darkness following the administration of hormone. Thus in conclusion it may be suggested that light plays an important role in mediating the reproductive effect of acute dose of melatonin. Moreover, this acute injection model may aid in better understanding of the mechanism of action of light on pineal activity and thereto the regulation of gonadal functions in higher vertebrates.

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