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THE INFLUENCES OF ILLUMINATION, MELATONIN AND PINEALECTOMY ON TESTICULAR FUNCTION IN THE RAT

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THE pineal gland influences the reproductive functions of mammals. However, responses to removal of the pineal, most times assessed from weight changes of the sex and accessory sex organs, have been inconsistent and are usually slight in the rat. In some instances, the lack of response to pinealectomy may have been due to various factors such as, the age of animals at pinealectomy [1,2], the early ambient lighting conditions[3], the level of olfactory stimulation [4], or the method of caging the animals [5]. Furthermore, the nature of gonadal changes resulting from stimuli affecting pineal function may even differ between strains of a given species [6].

The link between pineal activity and the photoperiod has recently become apparent [7]. The restriction of light to rodents causes activation of the enzyme hydroxyindole-O-methyl transferase with increased biosynthesis of pineal indoles [8] accompanied by enhanced antigonadotropic activity of the pineal [9]. Species differences are also apparent with respect to light deprivation. Normal testicular function of male hamsters requires twelve hours of daily illumination [10] whereas exposure to total darkness is reported to be less harmful in rats [11,12]. The retardation of accessory sex organ growth in rats as a result of light-deprivation can be prevented either by pinealectomy or by sympathetic denervation of the pineal which appears to render the gland inactive [9,12].

Melatonin, an indole uniquely elaborated by the pineal gland, has been frequently tested for its effects on mammalian reproduction. The results of

these studies tend to confirm the inhibitory nature of pineal material on the gonadal system. Despite evidence of suppression of genital growth in rats by melatonin [13,14] conflicting reports have appeared concerning the action of melatonin on the gonads of the growing male rat [15,16]. The dose of melatonin also affects the nature of responses of the gonads [17,18]. Implantation studies have shown that pineal indoles exert antigonadal influences by way of the anterior pituitary. Median eminence and reticular formation implants caused suppression of pituitary gonadotrophins whereby melatonin and 5-hydroxytryptophol regulate leutinizing hormone and 5-methoxytryptophol and serotonin influence the synthesis or secretion of follicle-stimulating hormone [19,20].

While the various parameters or indices of gonadal function would suggest a role of the pineal in sex hormone production, there appears to be no evidence in the literature of such a relationship in the rat. The present studies were undertaken to examine the nature of gonadal responses of the prepubertal rat to alteration of environmental lighting, melatonin administration and removal of the pineal gland and the effects of these stimuli on the in vivo secretion of testicular testosterone.

Methods

For these studies, male Sprague-Dawley rats (National Research Council, Ottawa) were employed and all experiments began with animals of age 24-26 days (50-55 g body weight). The rats were maintained in individual wire-mesh cages in light-proof rooms under controlled conditions and were fed a regular diet (Purina) and water ad libitum. The ambient temperature was kept at 21 ± 1°C, humidity at 45% and the lighting was controlled electronically (two 46 inch Westinghouse F40CW fluorescent lamps). Three different lighting cycles were employed as follows: light:dark 12:12 hours with light on 06.00 -18.00 hours; light:dark 23:1 hours with lights on 01.00 - 24.00 hours; light:dark 2:22 hours with lights on 10.00 - 12.00 hours. The light intensity at the level of the

cages averaged 76 foot-candles.

Pinealectomy was performed [21] on animals at 24-26 days of age using a specially designed [22] head-holder. Sham-operated controls were prepared in an identical manner except that the pineal gland was left in place. The melatonin solutions (500μg/ml) for injection were freshly prepared every third day by dissolving the material (Sigma Chemical Co.) in 1:1 ethanol:saline. All animals were injected intraperitoneally with 0.1 ml melatonin (50μg) solution 1-2 hours before the lights turned off each day of the experiment, and the control animals similarly received 0.1 ml 1:1 ethanol:saline.

The groups of animals in the pinealectomy and melatonin administration studies were maintained under a daily lighting cycle of light:dark (L:D) 12:12 hours. For the experiments involving the effects of altered illumination, animals were exposed to cycles of L:D 23:1 hours (extended illumination) and L:D 2:22 hours (light restriction).

At the end of a particular experimental period, animals were sacrificed by decapitation between 10.00 and 12.00 hours and the testes, seminal vesicles and prostate glands removed, cleaned free of extraneous tissue and weighed (Roller-Smith Precision balance). The weights of the seminal vesicles cleaned of fluid and of the ventral prostates were recorded. Weighed amounts of prostatic tissue were homogenized in distilled water using an all-glass homogenizer (Kontes). The protein was precipitated with cold 10% perchloric acid and, following centrifugation, the fructose content of the supernate was determined colorimetrically by reaction with resorcinol reagent [23] and measurement with a Pye Unicam 500 spectrophotometer.

For the determination of testosterone secretion rates, testicular vein blood was collected from animals anaesthetized with sodium pentobarbital (Nembutal, 25 mg/Kg body wt. i.p.) and heparinized (150-200 units) via the tail vein. A prominent branch of the testicular vein was located under the head of the epididymis of the exposed left testis [24] and a small slit was made in the vein with a No. 25 hypodermic needle. The exposed testis was immediately placed

in the neck of a pre-weighed 25 ml test tube and the animal rested on its abdomen. Blood was collected for a period of 15 minutes with the receptacle cooled by crushed ice and suspended directly below the animal. An internal recovery standard of 4000 dpm [4-^{14}C] testosterone (46 mCi/mmole, Amersham-Searle) and a volume of water equal to that of the blood sample was added and the contents of the tube were thoroughly mixed. Such samples were stored at -5^{o}C when not processed immediately.

Samples were extracted with ethyl acetate and analysed for testosterone content by double isotope assay [25]. Acetylations were carried out with 20% [^{3}H] acetic anhydride (100-500 mCi/mmole, Amersham-Searle) and, following thin-layer and paper chromatographic purification, the counting of labelled products was done on a Nuclear-Chicago Mark I liquid scintillation spectrometer equipped with an automatic external standard which was employed to correct for quenching. Counting was carried out in a toluene scintillator containing 4 g/litre PPO, 0.2 g/litre dimethyl-POPOP and 10 ml/litre ethanol and the efficiencies for tritium and carbon-14 were respectively 25% and 40% with the instrument settings employed.

Results and Discussion

Neither alteration of daily light cycle nor melatonin administration induced any significant change in body weight at any of the time intervals studied. In comparing the effects of short and long photoperiod, the data reveals a somewhat paradoxical effect at the 2-week time interval. While there occurred no significant difference between the weights of the seminal vesicles and prostate of the two groups of animals, rats subjected to the short daily photoperiod possessed smaller testes (745.3 ± 10 g/100 g B.W.) than those of the L:D 23:1 hr. group (836.7±25 g/100 g B.W.; $P < 0.02$) whereas the reverse situation appeared with respect to fructose content of the prostate (see Fig. 1) Decrease in testicular weight in response to extended darkness suggests decline in function at this early time period but is accompanied by an increase in accessory sex organ fructose which is indicative of enhanced testicular

steroidogenesis [26].

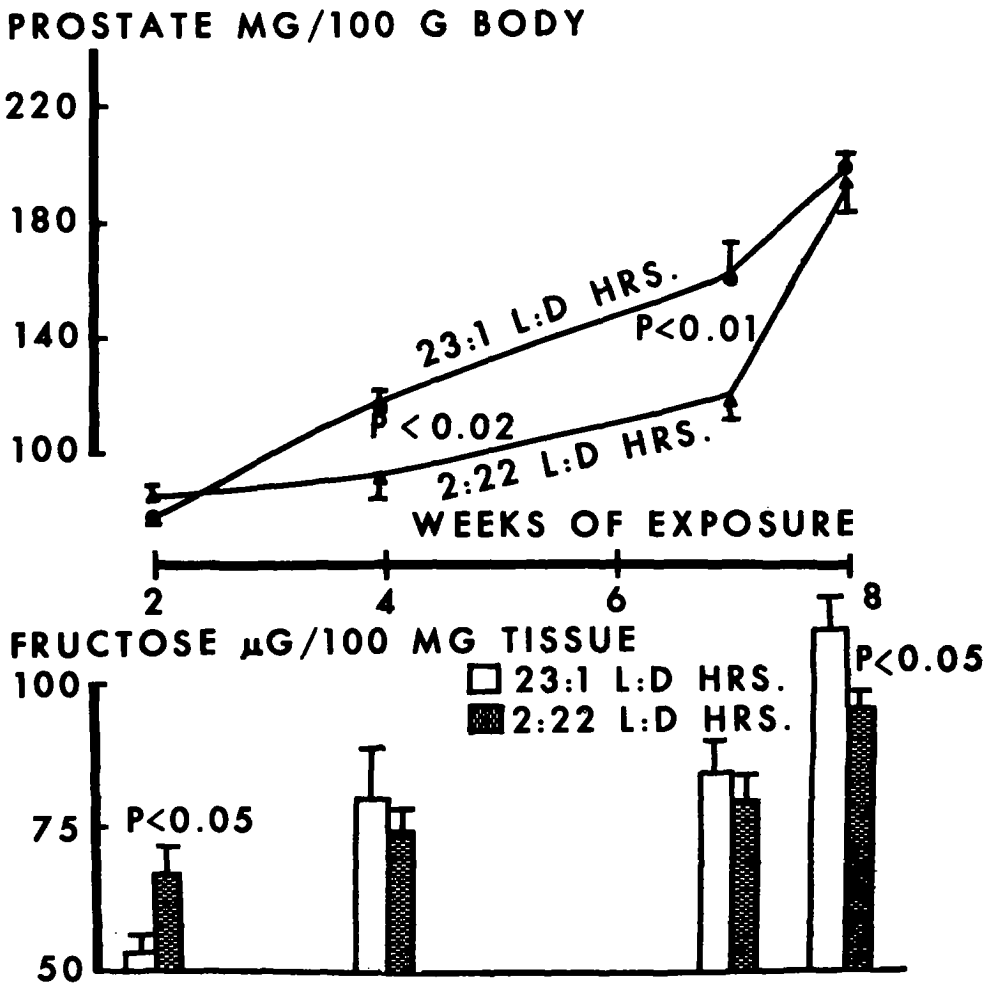

FIG.1

Weights and fructose contents of the prostate (mean ±S.E.) at various intervals of exposure to daily lighting regines.

No further changes in weight of the testes as a result of altered environmental illumination were noted at any of the subsequent time intervals of study. Smaller seminal vesicles of dark-exposed animals occurred (26.4 ± 4.2 vs 44.5 ± 1.8 mg/100 g B.W.) at the 4 week interval only. The weights of the prostate were significantly lower in the dark-treated animals both at 4 weeks

and 7 weeks after the beginning of the study but the difference had disappeared by 8 weeks. Depression in fructose content of the prostate ($P<0.05$) of animals exposed to the daily cycle L:D 2:22 hr. was apparent at this latter time interval.

The time course for the differences in prostatic weight and fructose between the two groups, L:D 23:1 and L:D 2:22 hr., is diagramatically illustrated in figure 1. The results show that after exposure to quite a wide difference in length of the daily photoperiod the magnitude of sexual response in the rat in terms of change in prostate weight is not very marked and is of relatively short duration, at least with this strain of animal. Alteration in fructose content of the prostate gland, an additional index of testicular steroidogenic function, illustrates the inhibitory influence of extended darkness but only at the 8-week interval and at a time when the differences in accessory sex organ weights have disappeared.

The daily administration of 50μg melatonin invoked no significant changes in the weight of the rat testes and induced significant reduction in weight of the seminal vesicles only after 8 weeks of treatment ($P<0.01$). In this instance, the weight of the seminal vesicles of melatonin-treated animals was 78.4 ± 3.3 mg/100 g B.W. compared to 98.4 ± 8.3 for ethanol:saline-treated controls. The nature of prostatic responses, shown in Figure 2, to melatonin were, in some respects, different to those seen as a result of altered environmental illumination. Whereas inhibition of prostatic growth was present at 4 and 6 weeks, both the magnitude of the differences between the two groups of animals was somewhat greater and an effect was still apparent at 8 weeks.

The inhibitory influences of melatonin on testicular function was demonstrable in terms of prostatic fructose content and, as was the case with altered illumination, significant change was manifested after changes in organ weight had begun, namely at 8 weeks.

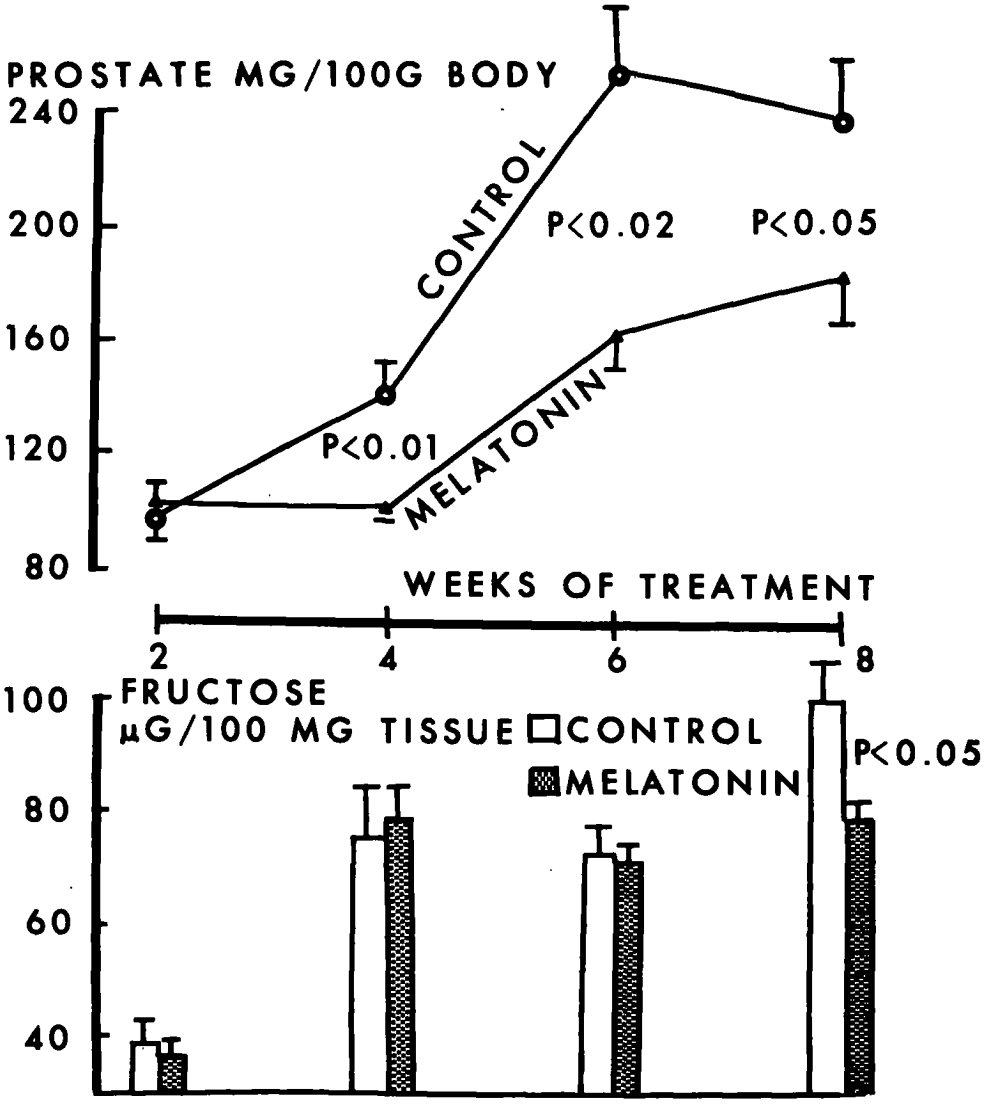

FIG. 2

Weights and fructose contents of the prostate (mean ± S.E.) at various time intervals of treatment with melatonin.

It was of particular interest to investigate changes in male hormone secretion by the testis and the time chosen for study was that when reflections in ventral prostate weight became evident at 4 weeks. The results, expressed in Table I, showed that significant increase in prostate weight in response to removal of the pineal gland occurred at this time interval. Restriction of light caused a dramatic reduction in testosterone secretion (2.53 ± 0.51

μg/100 ml blood) when compared with those of the L:D 23:1 group of animals (16.34 ± 2.27μg/100ml blood). These results are supportive of the findings in hamsters [27] where light deprivation led to lowering of plasma levels of testosterone as early as 12 days after onset of the lighting regimes. This effect was still evident 30 days later. The daily administration of melatonin caused suppression of testosterone secretion since the mean value for these animals (5.01 ± 1.20μg/100ml blood) was significantly lower than that of the ethanol:saline-treated rats (11.71 ± 1.21μg/100 ml blood). While these studies provide no information concerning the mechanism of melatonin-testicular interaction, the results fortify the previous indications [28] of an inhibitory action of melatonin on the synthesis of androstenedione and testosterone from labelled pregnenelone and progesterone by rat testicular preparations in vitro. In contrast to the effects of extended darkness and melatonin treatment, removal of the pineal gland led to an elevation of testosterone secretion in vivo when studied 4 weeks later. The mean level for pinealectomized control group was three times that of the sham-operated controls.

Table 1

Effects of Lighting, Melatonin and Pinealectomy on Testosterone Secretion.

Experiment+		Prostate wt (mg/100 g body wt.)	Testosterone Secretion (μg/100 ml blood)
1. Alteration of Environmental Light Cycle			
Light:Dark 23:1 hr	(6)	116.7±5.0	16.34±2.27
Light:Dark 2:22 hr	(7)	91.2±7.0	2.53±0.51
P (significance)		<0.02	<0.001
2. Daily administration of Melatonin 50 g i.p.*			
ethanol:saline	(5)	140.8±11.9	11.71±1.21
melatonin	(6)	100.8± 4.3	5.01±1.20
P (significance)		<0.01	<0.01
3. Removal of the Pineal Gland*			
Sham-pinealectomy	(7)	176.8±6.0	7.34±0.98
Pinealectomy	(9)	196.8±6.1	23.43±4.50
P (significance)		<0.05	<0.01

+experiments began with animals 24-26 days of age which were studied 4 weeks later. *animals were maintained under cycle L:D 12:12 hr. ( ) number of animals per group. Values are mean ± S.E.

These studies show that factors associated with or representing enhanced pineal function, i.e. exposure to extended periods of darkness and administration of melatonin, are inhibitory toward testicular function in the rat and cause suppression of testosterone secretion. The removal of antigonadal influences by pinealectomy leads to stimulation of testicular function and the elevation of production of testosterone

Summary

The results of these experiments suggest that indirect parameters such as changes in weight of sex tissues and accessory sex gland fructose are not always consistent and may, therefore, not adequately represent pineal-gonadal interrelations. Evaluation of testicular function, by direct measurement of secretion of the hormone testosterone, illustrated the proposed inhibitory actions of darkness and melatonin one month after the subjection of animals to the various stimuli, at a time when changes in ventral prostate weight were present but not maximal. The secretion of testicular testosterone was, however, markedly depressed by extended darkness at 4 weeks when compared with that found for rats exposed to a light cycle of L:D 23:1 hr. The treatment of rats with melatonin, produced a more pronounced inhibitory action on prostatic growth and effects were still operative at 8 weeks. In accordance with its reported anti-LH action, daily injection of melatonin lead to inhibition of testosterone secretion in vivo when examined after 4 weeks. The anti-gonadal nature of the pineal gland was further illustrated when its removal caused, in addition to stimulation of prostate growth, the enhancement of testosterone secretion rates 4 weeks after surgery.

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