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ORIGINAL ARTICLE

Melatonin inhibits testosterone secretion by acting at hypothalamo-pituitary-gonadal axis in the rat

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Abstract

OBJECTIVES: We have investigated the changes in serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone levels together with testicular histology in both pinealectomized (PNX) and intact rats. MATERIAL and METHODS: Twenty-one animals were PNX and allowed to recover for two months. Group I was assigned as PNX, group II PNX+melatonin and group III PNX+Human Chorionic Gonadotropin (HCG). Rats in group IV were sham PNX (S-PNX). An intact group of animals was s.c. injected with melatonin (0.5 mg/kg/day), another group with a combination of melatonin+HCG (5000 IU/kg/day) for seven days. Controls received saline alone (1 ml/kg). At the end, all animals were decapitated and blood samples obtained. Serum LH and FSH levels were determined by Radioimmunoassay, testosterone values by Chemiluminescent Enzyme Immunassay. Testicular tissue was collected and processed for light microscopy. RESULTS: Serum LH levels were increased following PNX, but no such increases were seen in testosterone. In the PNX+melatonin group, serum LH and testosterone values were found to be similar to those of S-PNX group. HCG supplementation to PNX rats resulted in significant decreases in LH ($p<0.005$), but increased testosterone levels ($p<0.001$). Melatonin administration to intact animals significantly decreased both LH and testosterone levels ($p<0.01$). Co-administration of HCG+melatonin resulted in significant decreases in LH ($p<0.001$) and increases in testosterone levels ($p<0.01$). Serum FSH values did not show significant changes among groups. Only HCG administration significantly reduced FSH levels ($p<0.01$). CONCLUSIONS: Our results suggest that melatonin inhibits testosterone secretion by acting at hypothalamo-pituitary axis. There is a functional relationship and feedback regulation between the pineal gland and the testes.

Introduction

It has been reported that the pineal hormone, melatonin, affects the male reproductive system. The data available in the literature suggest that melatonin has a negative influence on testicular functions [1]. It has been reported that melatonin secretion is increased in male patients with gonadotropin-releasing hormone (GnRH) deficiency and decreased to normal levels during testosterone treatment [2, 3]. Furthermore, nocturnal hypersecretion of melatonin in hypogonadal and infertile men with oligozoospermia or azoospermia and its normalization after testosterone propionate administration have been shown [4, 5].

These inhibitory effects of melatonin are primarily exerted through hypothalamo-pituitary axis [6, 7, 8]. Melatonin receptor sites have been visualized in the hypothalamus [9]. It has been reported that melatonin decreases GnRH-induced luteinizing hormone (LH) release from the neonatal, but not the adult, anterior pituitary gland [10]. This melatonin action is mediated by high-affinity membrane-bound melatonin receptors which are absent in adult rats. However, it has recently been suggested that melatonin could directly act at the gonadal level [11]. Melatonin may affect androgen secretion by direct action on the Leydig cells [12]. Niedziela & Lukaszyk [13] have shown that melatonin prevents testosterone release (induced by forskolin, a cAMP stimulator) in hamster Leydig cells. Presence of melatonin receptors on membranes of the rat Leydig cell has been shown [12]. These receptors also exist on the corpus epididymis in the rat [14].

Both receptor and non-receptor mediated mechanisms have been proposed for the actions of melatonin [15]. In the central nervous system, melatonin receptors are predominantly found in the nuclei [16]. However, melatonin action on the (neoanatal) rat pituitary LH release appears to be regulated by intracellular Ca2+ and cAMP-dependent mechanisms [8]. There is evidence that melatonin in gonadal tissues acts predominantly through membrane receptors coupled to adenylate cyclase [17].

There is limited information regarding melatonin action on follicle stimulating hormone (FSH) release and spermatogenesis in the male. In the female rat, melatonin has been shown to increase FSH secretion from the anterior pituitary on the day of pro-estrus [18]. Similarly, melatonin enhanced FSH response to GnRH in the follicular phase of menstrual cycle [19].

The present study was designed to examine the functional relationship between the pineal gland and hypophyseal-testicular axis. Serum LH, testosterone and FSH levels together with testicular histology were investigated in both pinealectomized (PNX) and intact animals.

Materials and methods

Adult male Wistar rats (Firat University Biomedical Unit, Elazig) in two different experiments were employed in this study. They were housed under controlled light (12 light and 12 dark) and temperature (21±1°C) conditions. Food and tap water were supplied ad libitum. In the first experiment, 21 animals were PNX and allowed to recover for a period of two months or longer. First group (n=7) was assigned as PNX. PNX rats in groups II (n=7) and III (n=7) received daily injections of melatonin (0.5 mg/kg/day, s.c.) or Human Chorionic Gonadotropin (HCG; 5000 IU/kg/day, s.c.), respectively, for seven days. Sham pinealectomy was (S-PNX, n=7) performed in the group IV.

In the second experiment, intact animals (group V, n=7) received daily melatonin administration (0.5 mg/kg/day, s.c.) or a combination of melatonin+HCG (group VI, n=7) for seven days. Controls (group VII, n=7) were given saline alone (1 ml/kg). At the end of the experiments, all animals were decapitated between 09.00 and 10.00 in the morning and trunk blood obtained. Blood samples were centrifuged for 10 mins at 3000 r.p.m. at 4°C. The sera were stored at –20°C until assayed.

Upon decapitation, testes were surgically removed and dissected from surrounding tissue. They were immediately fixed in 10% formalin solution for 24–48h and thereafter routinely processed for paraffin wax embedding. Sections were cut at 5 μm and stained with Haematoxylin and Eosin. The specimens were then examined by light microscopy.

Hormone assays: Serum levels of rLH and rFSH were determined by radio-immunoassay (RIA) following the instructions given with the reagents generously provided by the National Hormone and Pituitary Program of NIDDK (Baltimore, Maryland, USA). The rLH reference preparation was rLH-RP-2 and the antiserum was anti-rat LH-RIA-11. The sensitivity (90% B/Bo) of this assay using a 100 μl sample is about 0.16 ng/ml. The rFSH reference preparation was rFSH-RP-2 and the antiserum was anti-rat FSH-S-11. The sensitivity of this assay using a 200 μl sample is about 2 ng/ml. Both antigens were radiolabelled with iodine-125 (IMS 30 from Amersham Life Science Ltd, Bucks, UK). Bound and free radioiodinated antigens were separated using double antibody generously provided by the Scottish Antibody Production Unit, Law Hospital, Carluke, Lanarkshire, Scotland. The mean intra-assay coefficient of variation for two quality control samples was <5% in both assays.

Serum testosterone levels were measured by Chemiluminescent Enzyme Immunoassay (Diagnostic Product Corporation, Los Angeles, CA, USA).

Statistics: The hormone results were statistically analyzed by One-Way ANOVA (MINITAB, release 10 for Windows). Level of significance was set at $p<0.05$.

Results

Serum LH, FSH and testosterone levels are shown in Figures 1–3. Serum LH levels were significantly increased following pinealectomy compared to the S-PNX group. No such increases were seen in testosterone concentrations in the PNX group. In the PNX+ melatonin group, serum LH and testosterone values were found to be similar to those of the S-PNX group. HCG supplementation to PNX rats resulted in significant decreases in LH ($p<0.005$), but increased testosterone levels ($p<0.001$). Melatonin administration to intact animals significantly decreased both LH and testosterone concentrations compared to the control group ($p<0.01$). Co-administration of melatonin with HCG resulted in significant decreases in LH ($p<0.001$) and increases in testosterone levels ($p<0.01$). Serum FSH values did not show any significant changes among groups. Only HCG administration to intact rats significantly reduced FSH levels compared to saline-treated group ($p<0.01$).

Light microscopic examination of the control group testicular specimen is shown in Figure 4. Pinealectomy brought about increased mitotic activity in the seminiferous tubules (Figure 5). Number of type A and B spermatogonia adjacent to the basal membrane was increased. Primary spermatocytes with prominent nucleus were also increased in the seminiferous tubules. When exogenous melatonin was administered to PNX rats for a week, testicular histology was found to be similar to that seen in the S-PNX animals (Figure 6). Administration of exogenous melatonin to intact rats resulted in reduced spermatogenetic activity (Figure 7). Numbers of spermatids and spermatozoa were decreased.

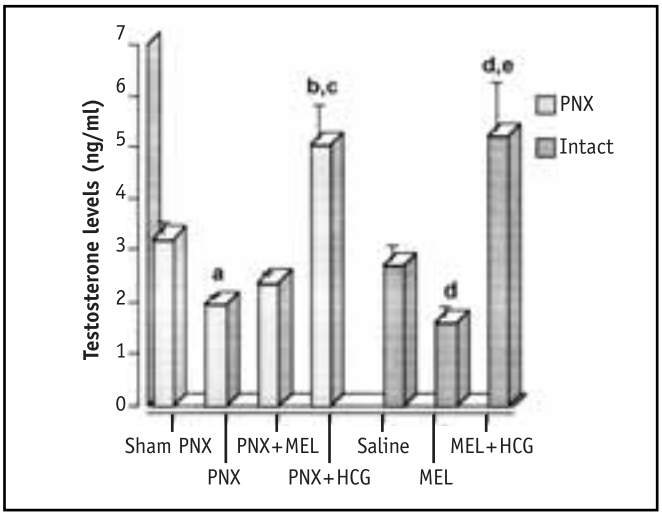

Fig. 2. Serum testosterone (ng/ml ± SEM) levels in the pinealectomized (PNX), S-PNX, PNX+Melatonin-treated, PNX+HCG-treated male rats, and also in intact animals following subcutaneous administration of saline, melatonin and melatonin+HCG combination for seven days. **a**: $p<0.005$; **b**: $p<0.01$ compared to S-PNX group, **c**: $p<0.05$ compared to PNX+MEL group, **d**: $p<0.05$ compared to saline- and **e**: $p<0.01$ compared to melatonin-treated animals, using One-Way ANOVA.

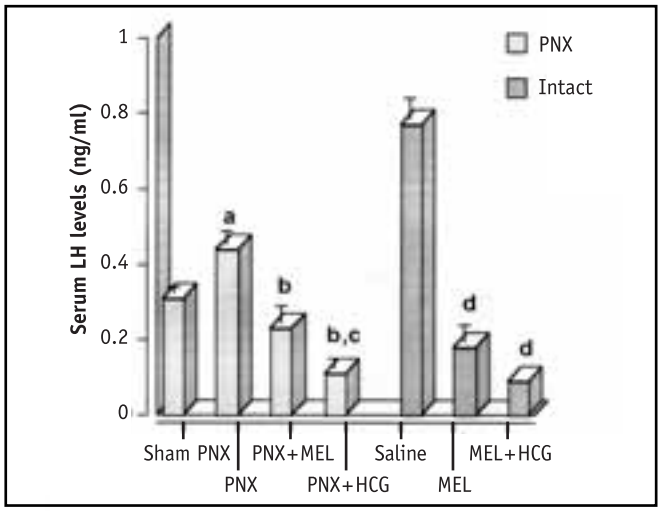

Fig. 1. Serum LH (ng/ml ± SEM) levels in the pinealectomized (PNX), S-PNX, PNX+Melatonin-treated, PNX+HCG-treated male rats, and also in intact animals following subcutaneous administration of saline, melatonin and melatonin+HCG combination for seven days. **a**: $p<0.05$, **b**: $p<0.005$ compared to S-PNX group, **c**: $p<0.001$ compared to the PNX and PNX+MEL groups, **d**: $p<0.001$ compared to the saline-treated animals, using One-Way ANOVA.

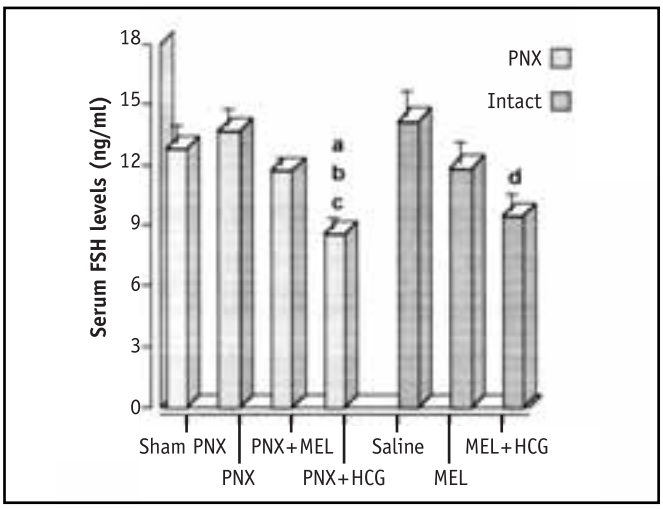

Fig. 3. Serum FSH (ng/ml ± SEM) levels in the pinealectomized (PNX), S-PNX, PNX+Melatonin-treated, PNX+HCG-treated male rats, and also in intact animals following subcutaneous administration of saline, melatonin and melatonin+HCG combination for seven days. **a**: $p<0.01$, **b**: $p<0.05$, **c**: $p<0.01$ compared to S-PNX, PNX and PNX+MEL groups, respectively. **d**: $p<0.05$ compared to saline-

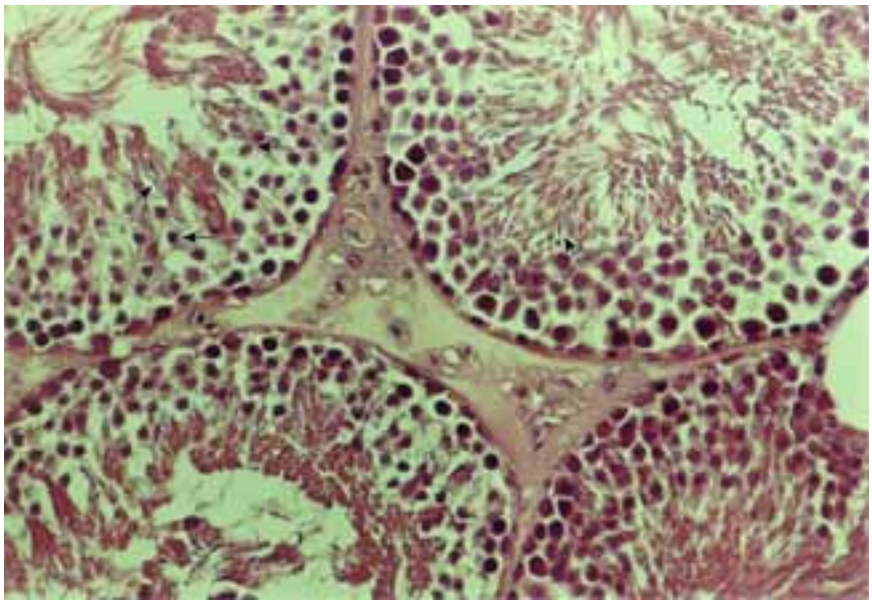

Fig. 4. Light microscopic visualization of testicular tissue in the sham-pienalectomized rats. Long arrow: spermatids, small arrow: spermatozoons. HE 20 X 5.

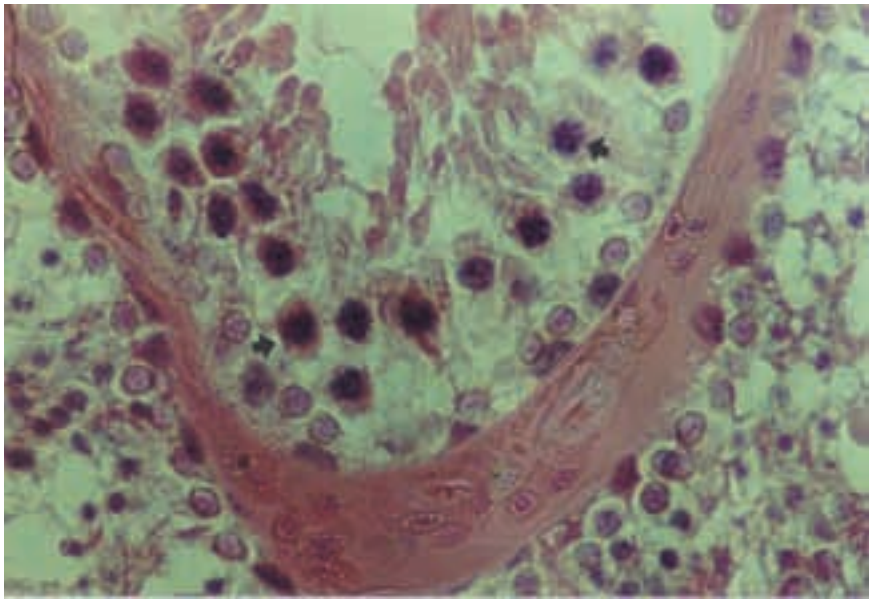

Figure 5. Light microscopic visualization of rat testicular tissue two months after pinealectomy. Large arrow: primary spermatocytes. HE 40 X 5.

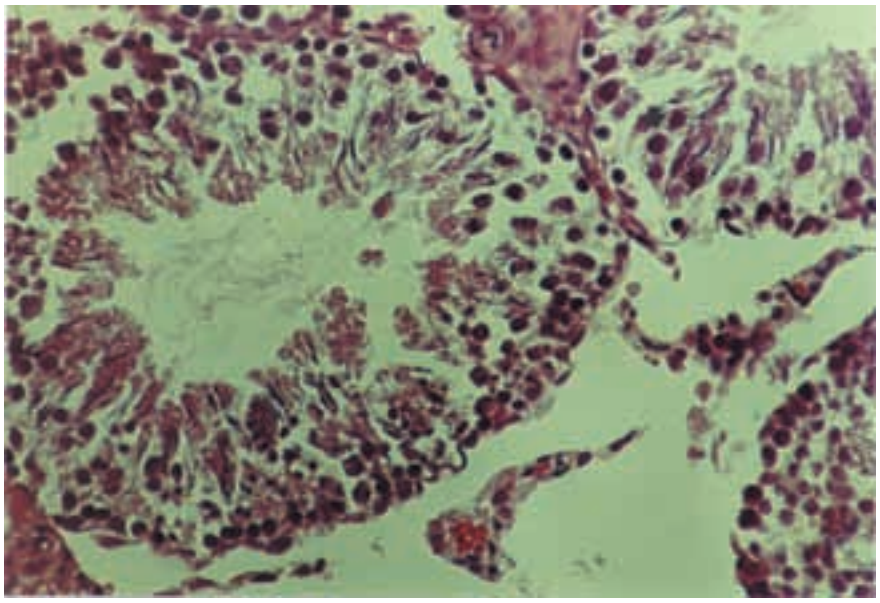

Fig. 6. Light microscopic visualization of testicular tissue following melatonin supplementation to the pinealectomized rats. Large arrow: increased meiotic activity in primary spermatocytes, long thin arrow: spermatids, short thin arrow: spermatozoons, HE 20 X 5.

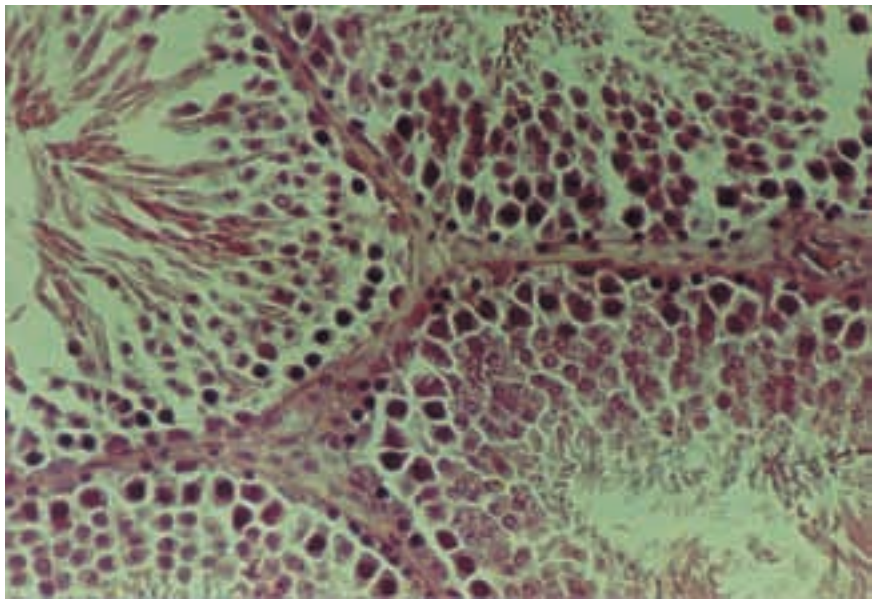

Fig. 7. Light microscopic visualization of testicular tissue following exogenous melatonin administration to intact rats for seven days. HE 20 X 5.

Discussion

In the present study, exogenous melatonin administration resulted in significant decreases in serum LH levels which were accompanied by reduced testosterone concentrations. It has previously been reported that melatonin receptors are not present on membranes of the gonadotropins [8]. Our results thus indicate that melatonin exerts its inhibitory effects through hypothalamo-pituitary-gonadal axis. Previously, existence of melatonin receptors on the rat Leydig cells has been shown [12, 20]. Either melatonin acts through the two different mechanisms to inhibit testosterone secretion or functionality of melatonin receptors in the testes await further investigation. In the PNX rats, serum LH levels were significantly increased; however, parallel rises in testosterone secretion were not seen. This was somewhat unexpected and this part of the experiment was repeated on a new group of PNX rats (n=6). The testosterone levels proved to be exactly the same as before. This seems to be an interesting finding, but difficult to interpret. Since the animals were allowed to recover for a period of two months, perhaps the Leydig cells desensitized to elevated levels of LH over this long period of time. Melatonin supplementation to PNX animals returned the serum LH and testosterone levels in parallel to those of the S-PNX group.

As expected, administration of HCG to PNX rats resulted in a significant increase in testosterone levels. Furthermore, HCG overcame the inhibitory effects of exogenous melatonin on testosterone secretion in intact animals. Significant reductions in serum LH levels in HCG-treated rats clearly indicate that elevated testosterone concentrations exert a negative influence on LH release.

A recent study has shown that melatonin administration markedly increases LH and FSH secretion in the pro-estrous rat [18]. Cagnacci et al. [19] have reported similar findings in women that melatonin enhances LH levels in the follicular menstrual phase. On the contrary, melatonin reduced naloxone-induced LH release in the ovariectomized and steroid-primed rat [21]. In the present study, although melatonin exerted a profound inhibitory influence on LH release, there was no significant variation in serum FSH concentrations either in PNX, PNX+melatonin-injected male rats or following exogenous melatonin administration to intact animals. Taken together, it is conceivable that there may be a sex-dependent difference for the direction of melatonin action on gonadotropin secretion.

The results of the present study have shown that LH secretion was profoundly suppressed by melatonin using two different animal models. However, serum levels of the other gonadotropic hormone, FSH, were not significantly altered in either of the animal models used. It is generally believed that gonadotropic hormone secretion is regulated by the hypothalamic decapeptide, GnRH. As explained above, the gonadotropins do not contain melatonin receptors in the adult rats [10]. Presence of a different hypothalamic FSH-releasing hormone (FSH-RH) has been proposed by McCann and his colleagues [22]. Differential regulation of LH and FSH by melatonin in our study thus provides indirect evidence for this hypothesis. However, negative feedback effects of increased testosterone levels on FSH release in the HCG-treated rats seem somehow controversial.

In the histological examination of the testes, signs

of increased mytotic activity were observed following pinealectomy, and these effects were reversed following melatonin supplementation to PNX rats. Administration of exogenous melatonin to intact rats resulted in reduced spermatogenetic activity. No precise observation could be made with regard to the interstitial Leydig cells under light microscopy. In our previous study, pinealectomy increased testicular weight and ultrastructural examination of the Leydig cells showed signs of hyperactivity [23]. These findings were reversed following melatonin supplementation for two months.

In conclusion, our results suggest that melatonin inhibits testosterone secretion by acting at the hypothalamo-pituitary-gonadal axis. It is thought that HCG-induced testosterone secretion is not affected by melatonin. Unlike an early suggestion by Ozata et al. [24], the present results indicate that there is a functional relationship and feedback regulation between the pineal gland and the testes.

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