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ULTRASTRUCTURAL INTERRELATIONSHIP BETWEEN THE PINEAL GLAND AND THE TESTIS IN THE MALE RAT

I. KUŞ
M. SARSILMAZ
M. ÖGETÜRK

Department of Anatomy, Fírat University Medical School, Elazi˘g, Turkey

B. YILMAZ
H. KELEŞTIMUR

Department of Physiology, Fírat University Medical School, Elazi˘g, Turkey

H. ÖNER

Department of Histology, Fírat University Medical School, Elazi˘g, Turkey

The ultrastructural interrelationship between the pineal gland and testis was evaluated in the rat. Wistar rats were divided into 6 groups. Groups I and II were sham-orchidectomized and orchidectomized rats, respectively. Rats in group III were orchidectomized and daily injected with testosterone propionate (TP) for 1 month. Groups IV and V were sham-pinealectomized and pinealectomized, respectively. Group VI was pinealectomized and daily injected with melatonin for 2 months. All animals were anesthetized with ketamine for fixation by vascular perfusion. Pineal glands of groups I, II, and III and the testes of groups IV, V, and VI were removed and weighed. All specimens were examined by electron microscopy. Orchidectomy caused an increase of lipid droplets, cytoplasmic dense bodies, and lysosomes. Rough endoplasmic reticulum, Golgi apparatus, and mitochondria were extensive in the cytoplasm. TP administration to orchidectomized rats resulted in formation of less extensive lipid droplets and mitochondria. In pinealectomized rats, golgi complex, mitochondria, and enlarged smooth endoplasmic reticulum were extensive in the cytoplasm of Leydig cells. Formation of cytoplasmic secretory granules and osmiophilic bodies was observed. Testicular weight increased compared to group IV. Melatonin decreased testicular weight in comparison to group V and prevented ultrastructural changes. Pinealectomy and orchidectomy caused hyperactivity in Leydig cells and pinealocytes, respectively, which suggests a mutual relationship between the pineal gland and testis in the rat.

Keywords electron microscopy, Leydig cell, melatonin, pinealocyte, testosterone

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Electron microscopic examination of the specimens collected was carried out at Gazi University, Medical School, Department of Histology and Embryology in Ankara, Turkey.

Address correspondence to Prof. Dr. Haluk Keleştimur, Fırat University, TiP Fakultesi (Medical School), Fizyoloji Anabilim Dali, 23119 Elazi˘g, Turkey. E-mail: hkelestimur@firat.edu.tr

Melatonin is released during the dark phase of the day by the pineal gland. In many mammals, the pineal gland, via circadian pattern of melatonin secretion, is involved in the regulation of the hypothalamo–hypophysial–gonadal axis [2]. Melatonin has inhibitory effects on both male and female gonads by direct (via melatonin receptors in reproductive organs) and/or indirect effects (via inhibition of luteinizing hormone [LH] release) mechanisms [18, 22]. However, the effects of melatonin on the male reproductive system has not been extensively studied [3]. Leydig cells are found singly and in clusters of various size within the interstitium of the testes [23]. They are large, polygonal, and acidophilic cells [19]. Cytoplasm of the Leydig cells contains abundant smooth endoplasmic reticulum, mitochondria, Golgi apparatus, and lipid droplets [19, 23]. Lipochrome pigment is frequently seen in these cells.

The pineal gland contains 2 types of parenchymal cells: pinealocytes and glial cells. The pinealocytes are the most common parenchymal cell in the pineal gland, which secretes melatonin. They have large, deeply infolded or lobulated nuclei that have prominent nucleoli [19]. The pinealocytes display an extensive cytoplasm that is variable in amount and contains membrane-bound granules, lysosomes, mitochondria, clusters of smooth endoplasmic reticulum, lipid droplets, and well-developed Golgi apparatus [4, 16]. Gonadectomy may affect both the function and ultrastructure of the pineal gland in various species [5]. There is an increase in the density of granular endoplasmic reticulum, Golgi apparatus, lipid droplets, lysosomes, and the number of ribosomes and mitochondria in the pinealocytes following castration [10, 20]. However, depressed pineal function after gonadectomy in the rat has also been suggested [21]. Pineal metabolism may also be modified by administration of gonadal steroids. Presence of androgen and estrogen receptors in the pinealocytes has recently been shown [8].

In the present study, we have examined the effects of melatonin on testicular weight and the ultrastructure of Leydig cells following pinealectomy in the rat. The effects of orchidectomy and orchidectomy followed by testosterone propionate (TP) replacement on the ultrastructure of the rat pinealocytes were also investigated.

MATERIALS AND METHOD

Adult male Wistar rats (weighing 180–200 g, $n = 72$) were kept under controlled temperature (21 ± 1°C) and photoperiod (07.00 to 19.00 h). Food (standard pellet diet) and tap water were supplied ad libitum.

The animals were divided into 6 groups. Group I ($n = 12$) and group II ($n = 12$) were designated as pinealocyte control (sham-orchidectomized) and orchidectomized rats, respectively. They received sesame oil (0.1 mL subcutaneously [sc]) alone. The rats in group III ($n = 12$) were orchidectomized and daily injected with testosterone propionate (TP; 0.5 mg/0.1 mL sesame oil per day sc; Sigma Chemical, Poole, UK) for 1 month commencing on day 7 after surgery. Group IV ($n = 12$) and group V ($n = 12$) were allocated as Leydig control (sham-pinealectomized) and pinealectomized rats, respectively. They received 10% ethanol (0.1 mL sc) alone. The animals in group VI ($n = 12$) were pinealectomized and daily injected with melatonin (3 mg/kg per 0.1 mL 10% ethanol sc; Sigma) for 2 months commencing on day 7 after surgery.

All animals were anesthetised with ketamine (100 mg/kg im) for fixation by vascular perfusion (2.5% glutaralaldehyde in 0.2 M phosphate buffer, pH 7.4) at the end of the experiments. The pineal glands of groups I, II, and III and the testes of groups IV, V, and VI were removed.

The testes were dissected from the surrounding tissue and weighed out. All specimens were fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4) at 4°C. They were postfixed in phosphate-buffered 1% osmium tetroxide. After dehydration in acetone, the specimens were embedded in Epon 812. Thin sections were cut on ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a Carlzeiss-900 electron microscope.

RESULTS

Orchidectomy caused an increase of lipid droplets, cytoplasmic dense bodies, mitochondria, and lysosomes. Rough endoplasmic reticulum was enlarged. Golgi apparatus was more intensive than those in the pinealocytes of the control animals (Figure 1). TP administration to the orchidectomized rats resulted in formation of less extensive lipid droplets and mitochondria compared to pinealocyte ultrastructure of both sham-operated and orchidectomized rats. Concentrations of cytoplasmic dense bodies were also decreased compared to pinealocytes of groups I and II. Extensiveness of rough endoplasmic reticulum in the pinealocytes of TP-administered rats was similar to that in controls.

Within the testes of the pinealectomized rats, Leydig cells contained large, indented nuclei with peripherally disposed heterochromatin. Golgi complex, smooth endoplasmic reticulum, and mitochondria with tubular cristae were extensive in the cytoplasm. The smooth endoplasmic reticulum of these rats was found to be enlarged. Formation of cytoplasmic secretory granules at various intensities and osmiophilic bodies were observed. The Leydig cells were similar to those seen in the control rats. In the pinealectomized rats, weight of the testicles increased compared to that of the control group. Daily melatonin administration resulted in significant decreases in testes weight compared to the pinealectomized rats (Table 1).

DISCUSSION

Feedback effects of the male gonads on function and ultrastructure of the pineal gland has been controversial [5, 15]. In this study, orchidectomy caused hyperactivity in the rat pinealocytes, which was refrained by administration of TP to the orchidectomized rats. In contrast, Cardinali and Vacas [6] suggested that melatonin synthesis in the pineal gland may decrease following castration and return to normal levels after testosterone treatment. Melatonin secretion increases in men with a deficiency of gonadotropin-releasing hormone (GnRH) and decreases to normal levels during testosterone treatment [11]. Nocturnal hypersecretion of melatonin occurs in hypogonadal and infertile men with oligozoospermia or azoospermia and normalizes after

Table 1. Mean ± SEM weight of the testes (gr/100gr body weight) in the control, pinealectomised and pinealectomised plus melatonin-injected rats

Groups	Mean ± SEM
Control (n = 12)	1.012 ± 0.002
Pinealectomised (n = 12)	1.107 ± 0.003*
Pinealectomised + Melatonin (n = 12)	0.997 ± 0.02**

*$p < 0.001$ compared to the control group.
**$p < 0.001$ compared to the pinealectomised group using One-Way ANOVA.
n = number of observations in each group.

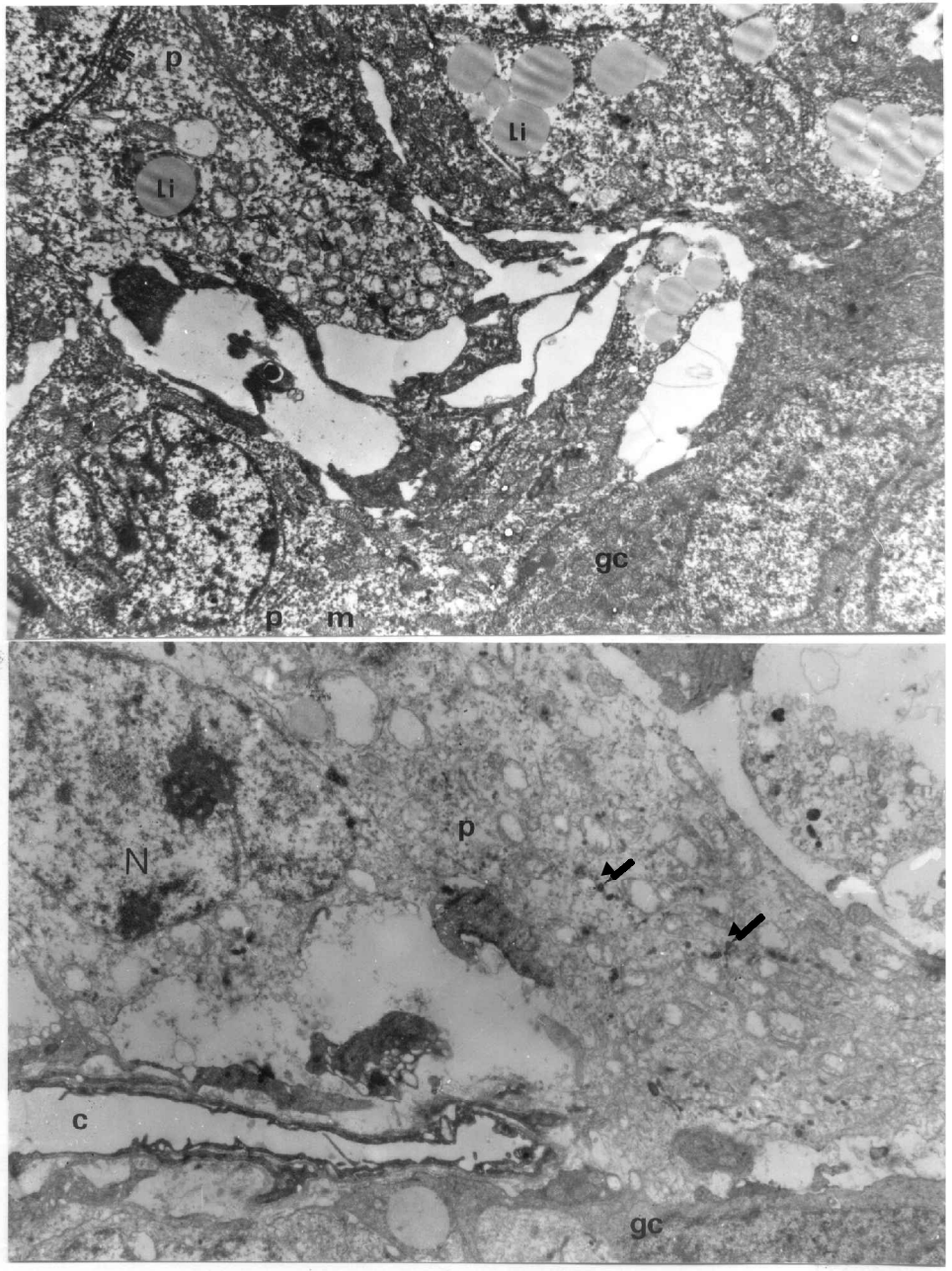

Figure 1. Electron micrograph of the pineal gland in the orchidectomized rats. *Top*: Orchidectomy caused an increase of lipid droplets (Li) and mitochondria (m). *Bottom*: Cytoplasmic dense bodies (arrows), ×3000.

TP administration [12, 14]. Surprisingly, exogenous GnRH administration did not suppress orchidectomy-induced hyperactivity in the rat pinealocytes [10]. In view of these reports and the present findings, we suggest that testosterone suppresses melatonin secretion and thus removes the inhibitory effects of melatonin on GnRH release or vice versa.

In the present study, pinealectomy caused significant increases in the testicular weight, which was reversed following chronic melatonin administration. A significant increase in the testicular weight 40 days after pinealectomy has been shown [7]. Melatonin injection caused a reduction in the diameters of seminiferous tubules and an inhibition of spermatogenesis in male golden hamsters [13]. Daily afternoon injections of melatonin to adult male hamsters for 50 days led to atrophy of the testes and accessory sex organs and a significant reduction in pituitary LH content and concentration [17]. Melatonin has an inhibitory influence on LH secretory mechanisms [1, 25]. Presence of melatonin receptors on the membranes of adult rat Leydig cells has been demonstrated [24, 27, 28]. Melatonin may also reduce sperm motility by its receptors on the sperm [26]. Leydig cells were decreased in size and contained reduced cytoplasm and shrunken and angular nuclei following melatonin administration in the hamster [13]. An increase in testicular weight and spermatogenesis was observed in pinealectomized male hamsters [9]. In agreement with these reports, the present ultrastructural findings show that pinealectomy brings about signs of interstitial hyperactivity in Leydig cells.

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