Effects of estradiol benzoate on the ultrastructure of the pinealocyte in the ovariectomized rat

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Abstract

OBJECTIVE: The aim of this study was to examine the effects of ovariectomy and ovariectomy followed by estradiol benzoate administration on the ultrastructure of pinealocytes in female rat.

DESIGN: For this purpose 15 female Wistar rats were used. Animals were divided into three groups. Group I and II were designated as sham-ovariectomized (control) and ovariectomized, respectively. Group III was ovariectomized and daily injected with estradiol benzoate for one month. At the end of the experiment, all animals were anesthetized with ketamine for fixation by vascular perfusion. Pineal glands of groups I, II and III were removed. All specimens were examined by electron microscopy.

RESULTS: Ovariectomy caused an increase of lipid droplets, mitochondria and ribosomes. Rough endoplasmic reticulum was extensive in the cytoplasm. Estradiol administration to ovariectomized rats resulted in formation of less extensive lipid droplets, mitochondria and ribosomes compared to pinealocyte ultrastructure of both control and ovariectomized rats. Extensiveness of rough endoplasmic reticulum in the pinealocytes of estradiol-administrated rats was similar to that in controls.

CONCLUSIONS: The results confirm relationship between the pineal gland and gonads in the rat and it has been suggested that estradiol benzoate reverses the ultrastructural changes, which indicate increased cell activation, occurring in the pinealocytes after ovariectomy.
Introduction

The mammalian pineal gland contains two types of parenchymal cells. The pinealocytes which form the majority of parenchymal cells and responsible for melatonin secretion in the pineal gland. Glial cells serve as supporting cells and they are fewer in number than pinealocytes [3, 5, 6, 12, 34].

Melatonin is released by the pineal gland during the dark phase of the day. In many mammals, the pineal gland is involved in the hypothalmo-hypophysyal-gonadal-axis by circadian pattern of melatonin secretion [1, 42]. It is generally accepted that melatonin has inhibitory effects on both male and female gonads by direct (via the melatonin receptors in the reproductive organs) and indirect (via inhibition of LH release) mechanisms [39]. However, gonadal steroid hormones change both the function and ultrastructure of the pineal gland in various species [7]. It has been reported that both gonadal steroid hormones and gonadotropins modulate melatonin secretion via specific pineal receptors [22]. Animal studies have demonstrated androgen and estrogen receptors in rat pinealocytes [13]. Additionally presence of androgen and estrogen receptors in the human pinealocytes has also been shown [22].

The results of the investigations concerning the effect of gonadal steroid hormones on the pineal gland are controversial. Several morphological and biochemical studies indicate an increased activity of pinealocytes following gonadectomy and this increase was inhibited by administration of gonadal steroid hormones [11, 14, 16-18, 20, 29, 32, 33, 36, 41]. Furthermore, It has been reported that, high levels of melatonin have been found in women with stress-induced [2] and exercise induced hypothalamic amenorrhea [21]. Luboshitzky et al., [23] have shown that melatonin secretion increases in men with a deficiency of gonadotropin-releasing hormone (GnRH) and decreases to normal levels during testosterone treatment. However, Satodate et al., [37, 38] reported that depression in pineal functions after ovariectomy in the rat. Furthermore, biochemical findings demonstrate that the decrease in melatonin and protein synthesis in the pineal gland following gonadectomy in female and male rats is restored to the normal level following estradiol or testosterone treatment [7, 9, 10, 15, 26, 27, 35, 40].

In the present study, we have examined the effects of ovariectomy and ovariectomy followed by estradiol benzoate administration on the ultrastructure of the rat pinealocytes.

Material and Methods

Adult female Wistar rats (weighing 150–180 g, n = 15) were used in this study. They were kept at a constant temp [21 ± 1 °C] and controlled light conditions (light, 07.00–19.00). Food (standard pellet diet) and tap water were supplied ad libitum.

The animals were divided into three groups. Group I (n = 5) and Group II (n = 5) were designated as control (sham-ovariectomy) and ovariectomized rats, respectively. They received sesame oil (0.1 ml s.c.) alone. Rats in group III (n=5) were ovariectomized and injected daily with estrogen hormone (Estradiol Benzoate; 0.5 mg/0.1ml sesame oil per day s.c; Sigma, St. Louis MO, USA) for one month beginning at day 7 after surgery. Vaginal smears of animals in Group I were performed before being killed and the rats showing regular 5-day estrous cycles with vaginal estrus were used as control. All animals were anaesthetized with ketamine (100 mg/kg i.m.) for fixation by vascular perfusion (%2.5 glutaraldehyde in 0.2M phosphate buffer pH 7.4) at the end of the experiments. The pineal glands of the Group I, II and III were then removed. All specimens were fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4) at 4 °C. They were post-fixed 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. All specimens were examined in Carlzeiss-900 electron microscope.

Results

Sham-ovariectomized rats (control)

In the parenchyma of the pineal gland, the main two types of cell observed were pinealocyte and glial cells. These cells were differentiated according to the their nuclear staining characteristics. The pinealocytes were rather large and oval shaped. The nuclei of this cells were large and poor in chromatin. The cytoplasm of pinealocytes was clear. Their cytoplasm had mitochondria and ribosomes. Glial cells were polygonal and elongated. Their nuclei were stained dark because of chromatin intensity. Furthermore, cytoplasm was dark and mitochondria and lipid droplets were observed (Fig. 1).

Ovariectomized rats

Following ovariectomy distinct changes were observed in the ultrastructure of the pinealocytes. The nuclei of pinealocytes were enlarged. The amount of mitochondria and ribosomes were increased. The rough endoplasmatic reticulum showed a marked proliferation and it was enlarged. A great number of cells contained numerous cisternae of rough endoplasmatic reticulum. Furthermore, lipid droplets were increased in number and size. In some cells, groups of numerous lipid droplets were observed (Fig. 2 a, b).

Ovariectomized and Estradiol benzoate-administrated rats

Estrogen hormone administration to the ovariectomized rats resulted in formation of less extensive lipid droplets, mitochondria and ribosomes compared to pinealocyte ultrastructure of both sham-operated and ovariectomized rats. Extensiveness of rough endoplasmatic reticulum in the pinealocytes of estradiol-administrated rats was similar to that in controls (Fig. 3).
Discussion

It is reported that gonadal steroid hormones have influence on structure and functions of the pineal gland [8]. In morphological and biochemical studies, it is well documented that an increase in activity of pinealocytes after gonadectomy is observed and this increase was inhibited by injection of gonadal steroid hormones [11, 14, 16–18, 20, 29, 32, 33, 36, 41]. Despite the presence of clinical studies supporting this idea [4, 23–25, 30], studies with complete opposite results are also present [7, 9, 10, 15, 26, 27, 35, 38, 40].

In the present study, ultrastructural changes in pinealocytes indicating an increase in the activity of pinealocytes after ovariectomy and inhibition of this increased activity with estrogen injection were observed. In an electron microscopic study by Karasek et al [18], ultrastructural changes indicating increased pineal protein synthesis after gonadectomy in rats were observed. Similarly Pevet et al [32] have also reported increased pineal protein synthesis following gonadectomy. In a previous study performed on rats, we observed increase in number of mitochondria, lipid droplets and cytoplasmic dense bodies and extensive number of lysosomes in the cytoplasm of pinealocytes after orchidectomy [20]. In the present study, enlargement of pinealocyte nuclei, increased amount of mitochondria, lipid droplets and ribosomes in the cytoplasm were observed alongside with the presence of extensive rough endoplasmic reticulum sacs after ovariectomy. This ultrastructural changes observed after ovariectomy can be interpreted as a morphological equivalent of increased protein synthesis. Present findings indicating increased activity of pinealocytes after ovariectomy is coherent to studies mentioned before [18, 20, 32].

Similarly, Gusak [14] has shown increase of mitochondria, lysosomes and ribosomes in the cytoplasm of pinealocytes after orchidectomy. Additionally, Das Gupta [11] has reported that hypertrophy of pinealocytes after orchidectomy. Studies on pig have shown increase in volume of pinealocyte nuclei and cytoplasmic dense bodies following ovariectomy [33, 41].

It is necessary for synthesis of melatonin taking tryptophan from the blood circulation into the pinealocytes [4, 12]. Major part of the tryptophan is utilized for melatonin synthesis and the remaining part is utilized for protein synthesis [12]. Therefore increased protein synthesis following ovariectomy may be used as an index of increased melatonin synthesis.

When investigation was performed on pinealocytes of estradiol administrated group, there were ultrastructural changes indicating decreased activity of pinealocytes. In this group of animals mitochondria, ribosomes and lipid droplets in pinealocytes were decreased compared to the ovariectomized groups. Additionally the extensiveness of the rough endoplasmic reticulum sacs were also decreased compared to ovariectomized group.

In a study by Sahu and Chakraborty in rats [36] it has been shown that the hypertrophy of pinealocytes after ovariectomy was inhibited by estrogen administration. Injection of estrogen to the ovariectomized pigs decreased cytoplasmic dense bodies and lysosomal vol-
Fig. 2. Electron micrograph of the pinealocytes in the ovariectomized rats. 

\textbf{a}: Ovariectomy caused an increase of lipid droplets (Li) and mitochondria (m). Magnification, X3000. 

\textbf{b}: Rough endoplasmic reticulum (rER), mitochondria (m), lipid droplets (Li) and ribosomes (arrows) were extensive in the cytoplasm. N: nucleus; P: pinealocyte. Magnification, X3000. (Publisher's comment: 90% of original size)
umes in the pinealocytes [33]. Studies performed on female rats also indicate the inhibition of increased melatonin synthesis after ovariectomy by estrogen administration [17, 29]. In addition to these reports, Okatani et al [28, 31] have found a correlation between the decrease in serum melatonin level at puberty and increased level of estrogen. In a previous study we have shown decreased mitochondria, lipid droplets and cytoplasmic dense bodies in the pinealocytes by injection of testosterone propionate to the orchidectomized rats [20]. The effect of estrogen on pinealocytes observed in the present study are in agreement with the previous similar studies.

Clinical studies have shown that serum melatonin levels increased due to stress and exercise-induced hypothalamic amenorrhoea in woman [21]. Additionally, Okatani et al [30] have shown that daily oral estrogen intake caused decrease in serum melatonin levels in postmenopausal women. Furthermore, in male patients with deficiency of GnRH or gonadotropin release and infertile male patients with oligozoospermia or azoospermia nocturnal melatonin secretion has been shown to be increased [19, 23, 24]. In these cases blood melatonin levels have been shown to decrease to the normal values by testosterone treatment [23, 25].

On the other hand, there are studies with controversial findings to the our study that are indicating inhibited pineal gland functions after gonadectomy which were stimulated by gonadal steroid hormones [7, 9, 10, 15, 26, 27, 35, 40]. Satodate et al [37, 38], have shown inhibited pinealocyte cell activation in gonadectomized rats. It is also reported that the activity of pinealocyte lysosomal enzymes was decreased after orchidectomy in hamsters [40]. In a biochemical study by Nagle et al [27] using male rats the activity of pineal HIOMT (hydroxyindole-O-methyl transferase) enzymes was shown to be decreased after orchidectomy which was recovered by injection of testosterone propionate. In another study, Nagle et al have also shown that [26], testosterone propionate administration following orchidectomy caused increased pineal protein synthesis. Similar effects were also reported by Cardinali et al [7, 9, 10]; in male and female rats the synthesis of pineal protein and melatonin was decreased following gonadectomy which was reversed by administration of estrogen and testosterone hormones.

In conclusion, an increase in pinealocyte cell activity after ovariectomy may be inhibited by injection of estradiol benzoate.

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