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Testicular Steroidogenesis after Pinealectomy - the Role of BDNF Signaling System

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The relationship between melatonin and steroidogenesis in the male reproductive system has been confirmed in many studies. However, there is limited data on changes in testicular steroidogenesis as an age-related process in melatonin deficiency conditions. In this regard, the immunohistochemical localization of the neurotrophic factor BDNF and its corresponding TrkB receptor was investigated in the Leydig Cells (LCs) of 5-, 16- and 20-month-old Wistar rats, divided into two groups – animals with removed pineal glands and SHAM-group. The results showed more pronounced immunoreactivity for BDNF and its receptor in the LCs of animals with removed pineal glands, compared to the SHAM group, as the expression correlates with age-related changes in the three groups. The data obtained confirm the role of melatonin as a regulator of testicular function by influencing the hypothalamic-pituitary-gonadal axis, as well as the role of the BDNF-signaling system in auto- and paracrine control of testicular steroidogenesis.

Key words: Leydig cells, melatonin, neurotrophic factor, steroidogenesis

Introduction

The main secretory product of the pineal gland – melatonin – is the subject of research, not only for its role in the regulation of circadian rhythms, but also for its diverse anti-inflammatory, antioxidant and anti-apoptotic effects [2, 10]. Well known is its suppressive effect on the secretion of the two adenohypophysial gonadotropic hormones – luteinizing hormone (LH) and follicle-stimulating hormone (FSH), affecting the male hypothalamic-pituitary-gonadal axis [1]. It has been established that by binding to its specific receptors, melatonin can directly regulate testosterone

secretion [13], and Frungieri et al., [1] demonstrated that melatonin can downregulate the expression of key steroidogenic enzymes.

Recently, an object of research has been the potential role of the BDNF-signaling system, consisting of the brain derived neurotrophic factor (BDNF) and its corresponding receptor TrkB, outside the development and proliferation of neurons, namely: in the autocrine and paracrine control of testicular steroidogenesis [11,12]. The expression of this signaling molecule and its corresponding receptor in testicular Leydig cells (LCs) supports the hypothesis that neurotrophins produced and/or acting on LCs are involved in the process of their differentiation and functional activity [3-8].

The immunohistochemical analysis of BDNF and its corresponding receptor TrkB, as markers of the differentiation status and activity of LCs in an experimental model of removed pineal gland makes it possible to clarify the action of melatonin on the processes of steroidogenesis in the male reproductive system during aging. In this regard, we aimed to investigate the immunoexpression of BDNF and its receptor TrkB in the LCs of rats in melatonin deficiency conditions.

Matherials and Methods

Male Wistar rats, bred under standard conditions (Temp.: 21 ± 1°C, humidity: 50-60% and artificial lighting mode) from three different age groups: 5 months (sexually mature), 16 months (adults) and 20 months (old), were divided into two groups: (n=8) – sham- and rats with removed pineal glands. The experimental model of pinealectomy was performed according to the methodology described by Hoffman and Reiter. Testicular fragments (4-5 mm thick) were gathered after decapitation of the animals and were fixed in Bouin's fluid for 24 h, embedded in paraffin and prepared for immunohistochemistry. The expression of BDNF and TrkB was monitored using the ABC method with the ImmunoCruz™ goat ABC Staining System kit (Santa Cruz Biotechnology, Inc., USA), with DAB as a chromogen and monoclonal primary anti-BDNF- and anti-TkrB antibody (1:100). Sections in which the primary antibody was substituted with phosphate buffered saline (PBS) were used as negative controls. The study was conducted in accordance with all accepted ethical guidelines for working with experimental animals and with the acknowledgement of the Scientific Ethics Committee at MU-Plovdiv No P-1583/2021.

Results

Our results demonstrate stronger imunoexpression of BDNF (**Fig. 1 A**) and it's TrkB-receptor (**Fig. 1 B**) in 5-month-old rats with removed pineal glands, compared to the SHAM-group (**Fig. 1 C** and **1 D**).

In 16-month-old rats, the immune response to BDNF in Leydig cells of the testicular interstitium was moderate. Again, the immune response is better expressed in animals with removed pineal gland (**Fig. 2**)

In 20-month-old animals, atrophic populations of LCs demonstrate relatively poor immunohistochemical reactivity for BDNF and TrkB in both groups – rats with removed pineal glands and SHAM – group (**Fig. 3**).

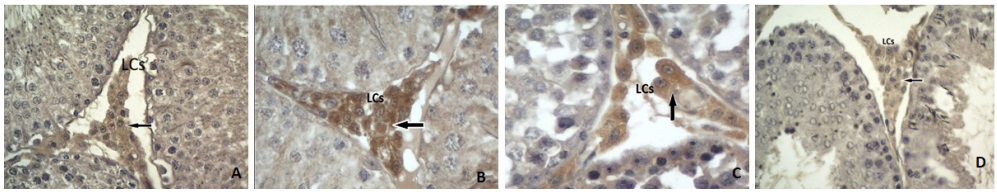

Fig. 1. Immunoreactivity for BDNF and TrkB in rat testis at 5 months postpartum. ×400 A and B – rats with removed pineal glands; C and D – SHAM-group

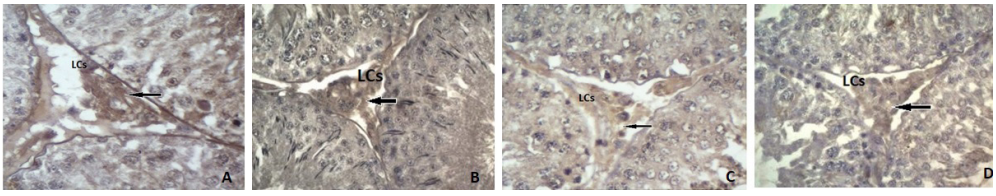

Fig. 2. Immunoreactivity for BDNF and TrkB in rat testis at 16 months postpartum. ×400 A and B – rats with removed pineal glands; C and D – SHAM-group

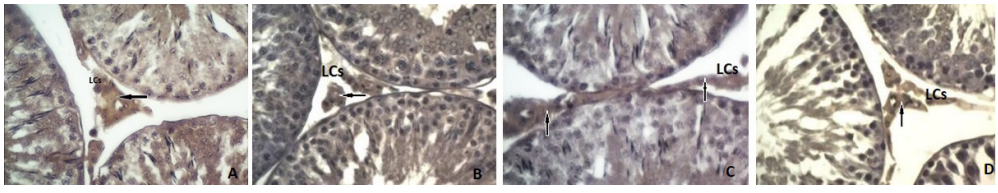

Fig. 3. Immunoreactivity for BDNF and TrkB in rat testis at 20 months postpartum. ×400 A and B – rats with removed pineal glands; C and D – SHAM-group

Discussion

Our study showed strong immunoreactivity for BDNF and its corresponding receptor TrkB in LCs of 5-month-old rats and a significant reduction in immunoexpression in the atrophic LCs populations of adult rats. In all three age groups, the immune reaction was better manifested in the animals with removed pineal glands. The gathered experimental data demonstrate the role of melatonin as a regulator of testicular steroidogenesis by influencing the male hypothalamic-pituitary-gonadal axis [1, 13]. These observations define the BDNF signaling system primarily as a paracrine factor with a leading role in LCs differentiation and functional activity and suggest the role of melatonin in the control of the testicular steroidogenesis under the regulatory effect of the hypothalamic-pituitary axis [1,10].

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