Institute of Experimental Morphology and Anthropology

Acta morphologica et anthropologica, 7
Sofia • 2002

Brain-derived neurotrophic factor (BDNF) and its receptor trkB in rat Leydig cells during the postnatal development

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The present study was designed to give an immunohistochemical evidence for the presence of the brain-derived neurotrophic factor (BDNF) and its corresponding receptor trkB in rat Leydig cells during the postnatal development.

On sections of Wistar rat testes at different stages of postnatal development (on postnatal days 5, 10, 15, 20, 24 and 27) the immunocytochemical expression of BDNF and its receptor trkB was studied.

Using an amplification immunocytochemical technique BDNF and trkB were visualized in the Leydig cells during all examined stages with characteristic fluctuation of labelling intensity. Also the Sertoli cells and some of the germ cells showed moderate to low immunostaining for the antigens studied.

The results obtained strengthen the view for the biological activities of the neurotrophic factors and suggest their role in the differentiation processes of the Leydig cells.

Key words: rat testis, Leydig cells, BDNF, trkB.

Introduction

The neurotrophic factors or neurotrophins is a small group of growth factors with homologous structure and biological activity. To this group belong also the brain-derived neurotrophic factor (BDNF) and its specific tyrosine kinase receptor trkB [2]. The neurotrophic factors are very important for the proliferation, differentiation and survival of nerve cells in the central and peripheral nervous system [2]. There is evidence lately for the existence of the neurotrophins and their receptors in different non-neuronal cells, as well as in the cell components of the animal testis [8, 15]. Recently, the immunolocalization of the neurotrophic factors and their corresponding receptors were established in rat and human differentiating and mature Leydig cells (LC) suggesting that these factors are involved in the LC development and differentiation [5, 11].

It has been reported that during rat development two LC populations existed: testosterone-producing fetal type LC and postnatal LC [11]. The fetal LC originate prenatal from interstitial mesenchymal-like precursor cells or stem cells, one part of which transform into the fetal type LC, while others remain spindle shaped and differentiate after birth into the mature LC [10, 12]. The fetal type LC rapidly regress during the first three weeks after birth [12]. It should be noted that the adult LC derive not from the fetal type, but from the undifferentiated stem cells. The embryonic origin of Leydig stem cells is still disputable. Data accumulated lately provide evidence that the LC of different species (included human) express marker substances for nerve and neuroendocrine cells and raise the question about the possible neuroectodermal (neural crest) origin of the Leydig stem cells [1, 7].

The purpose of the present study was to establish immunoreactivity for the BDNF and its receptor trkB in rat LC during the postnatal development.

Material and Methods

As material, testes of Wistar rats (n=16) on days 5, 10, 15, 20, 24 and 27 after birth were used. The experimental animals were killed under ether narcosis.

Immunocytochemistry

Paraffin sections (6 μm thick) fixed in Bouin's fluid were mounted on chrome-gelatin precoated slides. After incubation with 1.2% H_2O_2 in absolute methanol to inhibit endogenous peroxidase activity, the sections were treated with 2.0% normal swine serum (NSS, Sigma, Germany) to block non-specific binding sites. After that the sections were incubated for 24 hours at 4°C in a humid chamber with the primary antibody, as follows:

1. polyclonal rabbit **anti-BDNF** antiserum (Boehringer, Ingelheim Bioproducts, Germany, diluted 1:1000)

2. polyclonal rabbit **anti-trkB** antiserum (Santa Cruz, USA, 1:100). For the visualization of the antigen-antibody reaction an amplification technique consisting of combination of the peroxidase anti-peroxidase (PAP) and the avidin-biotin-peroxidase complex (ABC) methods was used [6]. The peroxidase activity was then developed by means of the nickel-gluckoseoxidase technique [16].

As controls, sections were used in which the primary, secondary or tertiary antibodies were replaced by phosphate-buffered saline (PBS) or only the peroxidase activity was visualized.

Results

By immunocytochemical analysis, a strong immunoreactivity for BDNF was observed within the LC during all examined stages of rat postnatal development, except on postnatal day 20 when a decrease in the staining intensity was found. The staining product was represented by dark-brown to black granules in the cytoplasm of the positive cells. Differences in the staining intensity were seen between the individual LC and also between the adjacent LC groups. Moderate immunostaining for BDNF exhibited also the Sertoli cells and some of the germ cells — primary spermatocytes (**Fig. 1**). The rat LC during postnatal development showed immunoreactivity for the trkB with the same fluctuation in staining intensity as in the other previously described

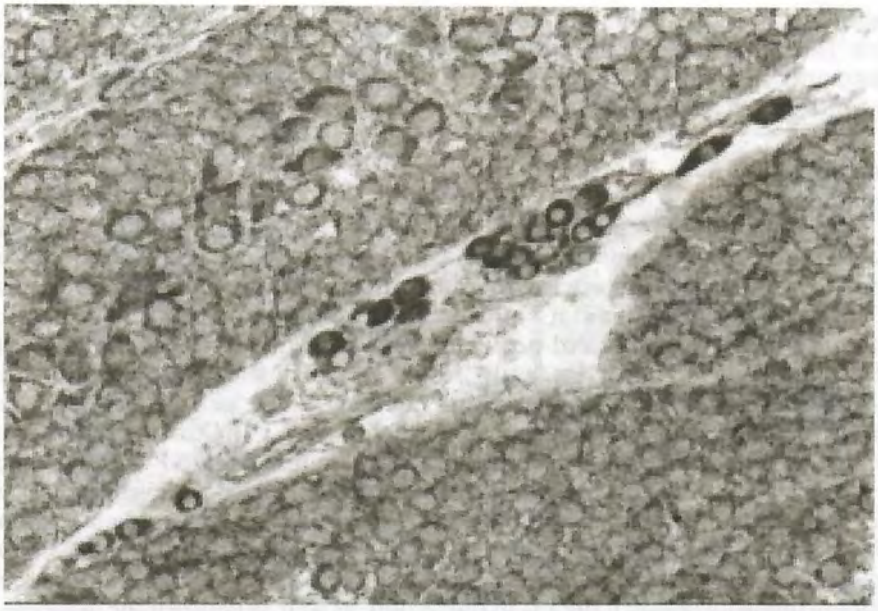

Fig. 1. Immunoreactivity for BDNF in a group of interstitial LC. Positive were also the primary spermatocytes (postnatal day 15) (×820)

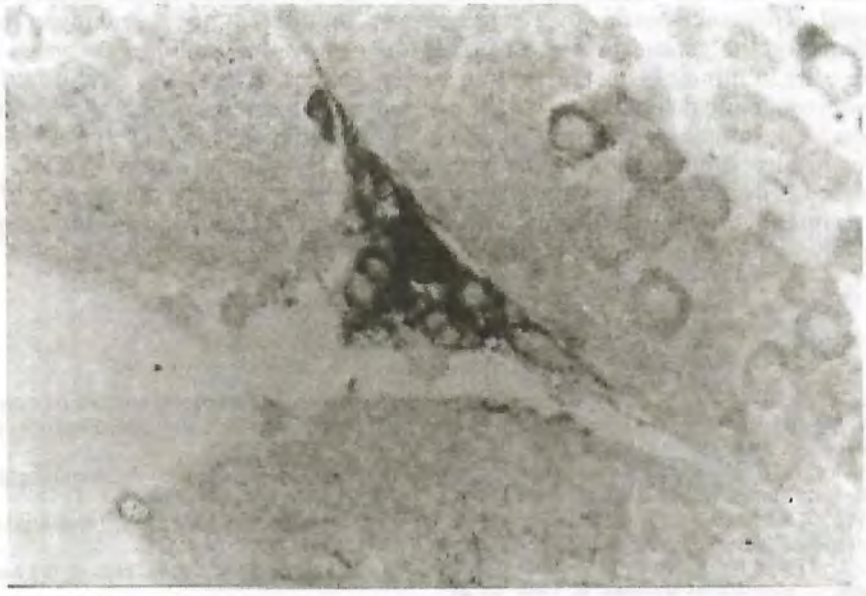

Fig. 2. trkB-immunoreactivity in the LC on day 24 postpartum. Positive signals in the germ cells (primary spermatocytes) (×820)

antigen. The lowest trkB immunoreactivity was seen in the LC at day 20 postpartum. Positive for the high affinity receptor trkB were also some of the germ cells predominantly the primary spermatocytes (**Fig. 2**). No specific reaction was detected in the control sections (*not shown*).

Discussion

It has been shown that there are three distinct stages in the postnatal development of the rat LC: progenitors, immature type and adult type LC [10]. The differentiation of mature LC is a continuous process of transformation of progenitors into the adult type. The Leydig cell progenitors arise from mesenchymal-like stem cells of the interstitial tissue during the third postnatal week [9]. The origin of the Leydig stem cells lineage is still unknown. Recently, results obtained showing the expression of numerous marker substances for nerve and neuroendocrine cells in the Leydig cells support the hypothesis for their neuroectodermal origin [1, 4]. Our study revealed the immunocytochemical expression of the neurotrophic factor BDNF and its receptor trkB in rat LC during all examined stages of postnatal development with characteristic fluctuation in staining intensity. The observed labelling pattern could be referred to the change of rat LC populations — prenatal and postnatal and with their different stage of differentiation and functional activity [10]. The differences in the staining intensity between the LC in one group, as well as between the adjacent LC group were associated with the heterogeneity of LC populations in rat postnatal testis [3]. Immunoreactivity for the examined antigens was found also in the Sertoli cells and in the primary spermatocytes. These findings suggest that the neurotrophic factors may act on LC differentiation via auto-and/or paracrine fashions. Our results are in concordance with the accumulated evidence that the neurotrophic factors have functional significance outside the nervous system, in different peripheral target tissue [14]. Recently, the gene-expression of trkB was observed in some of the germ and somatic cells in mouse testis [13]. In our earlier studies, it has been established the immunocytochemical localization of some neurotrophic factors and their specific receptors in developing and adult rat and human Leydig cells [5, 11] .

The results obtained showing the immunocytochemical expression of BDNF and trkB in rat LC during the postnatal development provide evidence for the role of the neurotrophic factors in the differentiation processes of the Leydig cells and support the hypothesis for their neuroectodermal origin.

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