Original Article

EXPRESSION OF NEUTROPHIN 4 IN POSTNATAL RAT RETINA

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Summary

Many types of neurons in central nervous system are excited by brain derived neutrophin factor (BDNF) and Neutrophin 4 (NT-4), an action that has recently been implicated in synaptic plasticity. NT-4, presumably activated via TrkB, selectively control the differentiation of distinct ganglion cell neurite morphologies. We have studied the expression of the NT-4 in the developing rat retina on postnatal days 1, 3, 5, 7, 9, 11, 16, 30. Changes in expression of NT-4 and localization in developing retina were assessed by immunohistochemistry method.

During early retina's development NT-4 is localized in ganglion cells, inner plexiform layer (IPL), displayed ganglion cells in IPL, inner parts of ventricular layer and nerve fibers (NL). The expression of NT-4 in outer segments of photoreceptor cells begins at about day 7. At postnatal day 9 horizontal and bipolar are already well marked. The distribution of NT-4 in development rat retina suggests that it plays role in neurite outgrowth, differention, survival of a kind of cell population and synaptogenesis of internal part of postnatal rat retina. The expression and role of NT-4 in outer segment of photoreception cells are to be discussed in some future researches.

Key words: retina, neutrophins, NT-4, development

Introduction

Neutrophins are polypeptide molecules that belong to a family which comprises six closely related factors: nerve growth factor (NGF), brain derived neutrophin factor (BDNF), Neutrophin 3 (NT-3), Neutrophin 4 (NT-4), Neutrophin 6 (NT-6) and Neutrophin 7 (NT-7), a member of the neutrophin family(found so far only with the Zebrafish). It can be made a distinction between two subtypes of neurotrophin receptors: the first consists of the tyrosine kinase (Trk) family of receptors,known as TrkA, TrkB and TrkC, which specially bind individual neurotrophins; the second type is low-affinity neutrophin receptor p75, which binds neutrophins with varying affinities and does not posses intrinsic tyrosine kinase activity [1]. Local application of NT-4 prevented the death of 45% of facial neurons after sectioning in neonatal rats. The survival number of corticospinal motor neurons cultured in vivo depended on the content of NT-4 in the culture medium [2]. NT-4 was 3 orders of magnitude less potent than BDNF as survival factor for early dorsomedial trigeminal sensory neurons during the phase of naturally occurring death [3].

We investigated the expression of the NT-4 in the postnatal developing rat retina.

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Materials and Methods

Animals and tissue preparation

Wistar rats of postnatal days 1(P1), 3 (P3), 5 (P5), 7 (P7), 9 (P9), 11 (P11), 16 (P16) and 30 (P30) were obtained from the Medical University of Pleven Vivarium and after anesthesia with ether were decapitated. Eyes were enucleated, briefly fixed in Carnoa, and after the removal of the anterior segment the eyes were returned to fixation for 2 hr. According to the routine method fixated eyes were embedded in paraffin and 4-5 µm sections were cut on microtome.

Immunohistochemistry

Deparaffined sections were incubated overnight at 4^{0}C with a 1:100 dilution rabbit polyclonal antirabbit NT-4 antibody. The ABC kit (Santa Cruz Biotechnology, California) was used to visualize the NT-4 antibody by using the peroxidaze-3-3′-diaminobenzidane (DAB) reaction according to the manufacturer's instructions.

Results

The differentiation and definite localization of rat retinal cell populations begin at the embrionic period and continue during the first four week after bird. On the first postnatal day the retina consists of two neuroblast layers: inner and outer which are separated by newly formed inner plexiform layer (IPL). The inner cellular layer is arranged by bodies of ganglion cells which begin their differentiation in embryonic development. The outer layer divides out of the scleral near to the pigmental layer and ventricular in which the photoreceptors (FC), horizontal (HC), bipolar (BC) and amacrine (AC) cells begin their differentiation. The outer plexiform layer (OPL) is formed between P5-P12 by terminations of FC, HC and BC.

At P1 the bodies of ganglion cells (RGC) were clearly seen. The ganglion cell layer (GCL) was composed of multiple layers at P0 and P5, but its thickness decreased and it became a monolayer by P17. Fine positive reaction showed IPL, displayed ganglion cells in IPL, inner parts of ventricular layer and pigmental cells (PC) (Fig. 1).

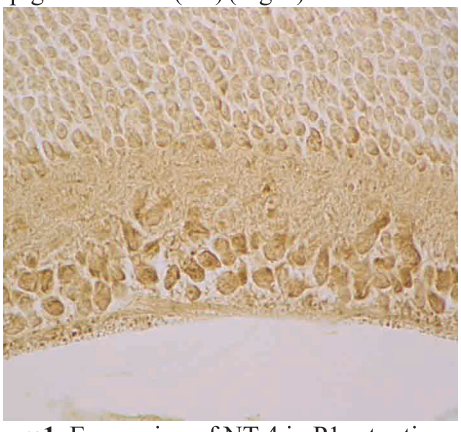

Figure1. Expression of NT-4 in P1 rat retina. Labeling for NT-4 is most prominent in GCL. Fine positive reaction is visible in IPL, displayed ganglion cells in IPL, inner parts of ventricular layer (Magnification x 400).

Between P3-P5 the AC, HC, the body of divided FC and nerve fibres (NL) were positive (Fig.2).

At P7 the fine immunoreactivity in cell membranes of INL and ONL, newly formed OPL, outer segments of photoreceptors and forming outer limited membrane (MLE) could be identified (Fig. 3).

The cell membrane of AC and BC in inner nuclear layer (INL) were well expressed at P9 (Fig. 4).

NT-4 expression increased in OPL, IPL and FL at P11 (Fig5).

At P16 and P30 strong reaction was seen in GC, OPL, IPL and OS (Fig. 6).

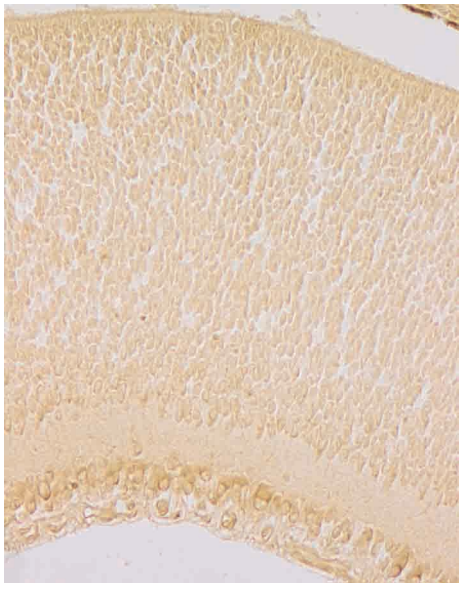

Figure 2. Expression of NT-4 in P5 rat retina. AC, HC, the body of divided FC and nerve fibres (NL) are positive (Magnification x 200).

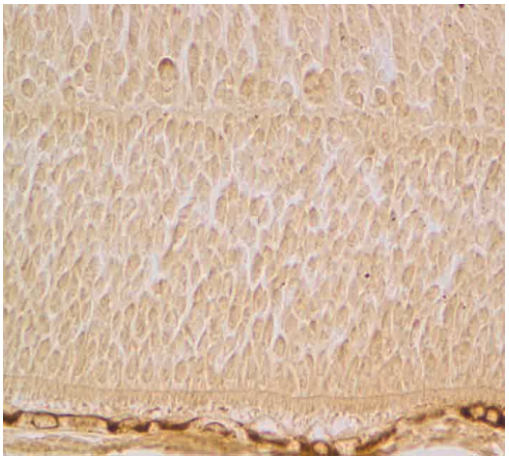

Figure 3. Expression of NT-4 in P7 rat retina. NGF immunoreactivity in the outer segments of photoreceptors and forming outer limited membrane (MLE) can be identified (Magnification x 400).

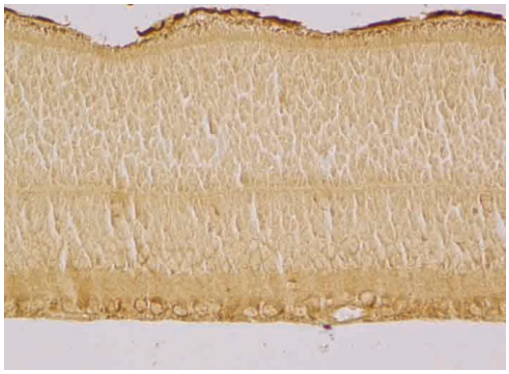

Figure 4. Expression of NT-4 in P9 rat retina. NGF immunoreactivity can be seen in the cell membrane of AC and BC in INL (Magnification x 400).

Figure 5. Expression of NT-4 in P11 rat retina. NGF expression increases in OPL, IPL and FL at P11 (Magnification x 400).

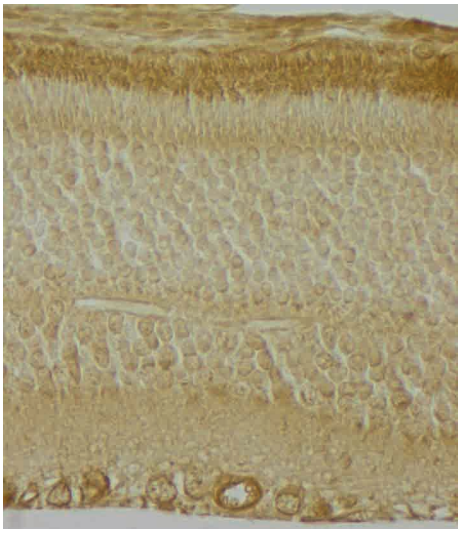

Figure 6. Expression of NT-4 in P30 rat retina. NGF immunoreactivity in RGL, IPL, OPL and 0S is well visible (Magnification x 400).

Discussion

The neutrophins brain derived neurotrophic factor (BDNF), neurotrophin 4 (NT-4) as well as their specific tyrosine kinase receptors (TrkB) are expressed in both the developing and adult visual system and they influence the proliferation, neurite outgrowth and the survival of cells in the visual system in vitro and in vivo [4]. BDNF and NT-4 have been been implicated in synaptic plasticity and regeneration of ganglion cells after axotomy or ischemia (5). We investigated the expression of NT-4 in postnatal rat retina as example of well blood-supplied retina.

Differention of RGC begins on E12 and finishes on P14. NT-4 was expressed in RGL on the first postnatal day and increased to the mature retina, suggesting that NT-4, presumably activated via TrkB, selectively control the differentiation of distinct ganglion cell. Our results are supported by previous researches. NT-4 was shown only to promote retinal outgrowth in vitro in presence of proliferating glia and play a secondary role in this process [6,7], but NT-4 was more effective than BDNF in neurite outgrowth of GC in short-term culture of postnatal rats. BDNF supported by predominantly polarized outgrowth, whereas NT-4 induced the appearance of intensely branched symmetrical arbors [4]. In vitro and in vivo studies suggest that BDNF and NT-4 can enhance the survival of developing, adult and injured retinal ganglion cells [8, 9, 10].

Our results showed expression of NT-4 in NF, IPL and OPL at the beginning of their formation accordingly on the P1 and P5-P7. Positive reaction was increased in the next week and was well visible in mature retina. The distribution of NT-4 in postnatal rat retina suggests that it plays role in neurite outgrowth and synaptic plasticity and formation during the postnatal development and degeneration. Injections of NT-4 during the second or third postnatal week caused increased labeling of dopaminergic fibers in lamina 1 and 3 in IPL in rodent retina [11]. The loss of support from NT-4 could potentially be a factor in degeneration and apoptotic cell death found in the retina in various pathologies [12]. The prolonged administration of NT-4 by mini-pump increased axon branch median lengths by eightfold but had no effect on the number of branches formed by the GC axons in the retinas of adult rats after optic nerve transection [13].

We observed the increasing positive reaction in outer segments of photoreceptors during the development. This finding is very intriguing, because outer segments of photoreceptors do not normally express TrkB except green-red-sensitive cone outer segments [14, 15, our investigations -not published] it is difficult to discuss these facts. May be NT-4 has a specific role in the function and maintenance of photoreceptors. The expression and role of NT-4 in outer segment of photoreception cells are to be discussed in some future researches.

Conclusion

The distribution of NT-4 in development rat retina suggests that it plays role in neurite outgrowth, differention, survival of some types of cell population and synaptogenesis of internal part of postnatal rat retina and take part in function and maintenance of photoreceptors.

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