Original Article

LOCALIZATION OF NERVE GROWTH FACTOR DURING POSTNATAL DEVELOPMENT OF THE RAT RETINA

Stilyanka D. Yochkova,
Dorka M. Vouchidolova,
Izkren K. Gerasimov

Department of Anatomy, Histology and Embryology
Medical University - Pleven

Summary
Nerve growth factor (NGF) is known to stimulate neurite outgrowth and support neuronal survival during retinal development and regeneration through the high-affinity tyrosine kinase (Trk) receptors. On the other hand, NGF binding to the low-affinity neurotrophin receptor p75 might induce programmed death in early phase of retinal development. We have studied the expression of the nerve growth factor in the postnatal development rat retina. We investigated NGF in retinas of rats on postnatal day 1, 3, 5, 7, 9, 11, 16, 30. Changes in expression of NGF and localization in developing retina were assessed by immunohistochemistry method. During early retina's development NGF is localized in ganglion cells, Muller and pigment cells and its expression increased during next days. Amacrine, horizontal and bipolar cells express NGF lately. The expression of NGF can be demonstrated in inner segments and external limiting membrane about day 5. The distribution of NGF in postnatal rat retina suggests that it plays role in neurite outgrowth, regulation of number of ganglion cells and synaptogenesis in inner retina. NGF take part in differentiation of inner segments of photoreceptor cells.
Key words: NGF, neutrophins, retina, postnatal

Introduction
NGF was the first neurotrophic agent to be discovered and has become paradigm of factors preventing cell death in selective populations of neurons during the development of central and peripheral nervous system. The trophic signals elicited by NGF are transduced through its specific tyrosine kinase receptor TrkA and modulated by the common neurotrophin receptor p75 [1].

We investigated the expression of NGF in the postnatal development rat retina.

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 a commercial source and after anesthesia with ether were decapitated. Eyes were enucleated, briefly fixed in Carnoa, and after removal of the anterior segment the eyes were returned to fixation for 2 hr. According to the routine method the fixated eyes were embedded in paraffin and 4-5 μm sections were cut on microtome.

Immunohistochemistry
Deparaffined sections were incubated overnight at 4^0C with a 1:100 dilution rabbit polyclonal antirabbit NGF

Corresponding Author:
Stilyanka D. Yochkova
Department of Anatomy, Histology and Embryology
Medical University - Pleven
1, ST. Kliment Ohridski str.
Pleven, 5800
Bulgaria
e-mail: mamasteli@abv.bg

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antibody. The ABC kit (Santa Cruz Biotechnology, California) was used to visualize the NGF 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 became during the first four weeks after birth. At P1 was clearly seen the multiple ganglion layer (RGL), displayed newly formed inner plexiform layer (IPL) and fine positive cells in the ventricular layer and processes of pigmental cells (PC) and blood vessels in nerve fibers (NL) (Fig.1).

The inner segments of photoreceptors, body's cones and newly forming outer plexiform layer (OPL) showed the positive reaction at P5 (Fig. 2).

The immunoreactivity for NGF was increased in cell membranes of INL and ONL, inner segments of photoreceptors and outer limited membrane (MLE) at P9 (Fig. 3).

At P11 NGF expressed in blood vessels in INL and well painting was visualized in horizontal cells (HC), bipolar cells (BC), inner segments (IS) and outer limited membrane MLE (Fig. 4).

At P16 and P30 strong reaction was seen in retinal ganglion cells (RGC), PC, IPL, some AC, MLE and IS

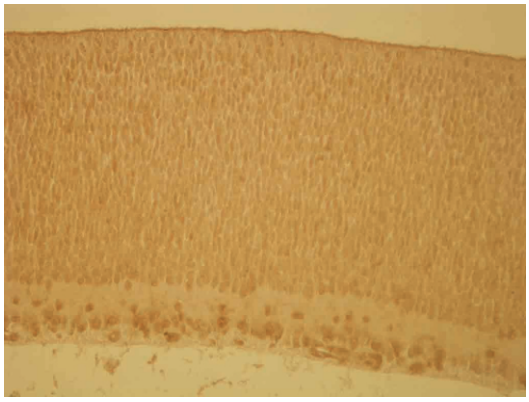

Figure1. Expression of NGF in P1 rat retina. Labeling for NGF is most prominent in GCL. Labeling is also detected in IPL, ventricular layer and pigmental cells (Magnification x 400).

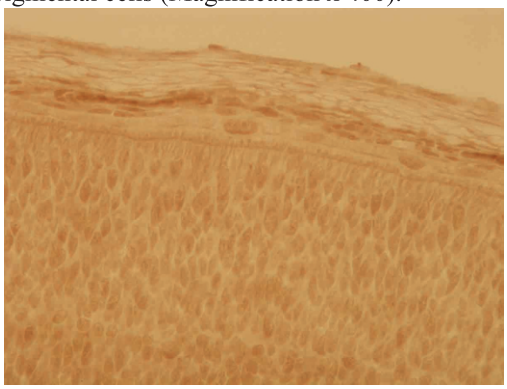

Figure 2. Expression of NGF in P5 rat retina. Inner segments of photoreceptors, body's cones and OPL are positive (Magnification x 400).

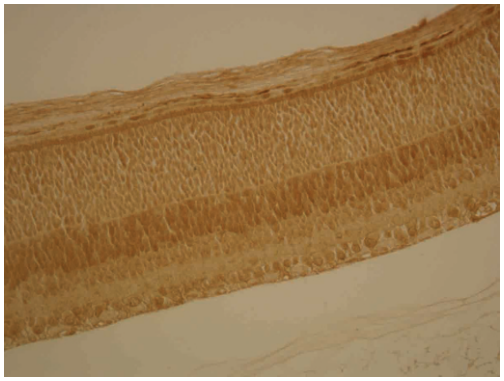

Figure 3. Expression of NGF in P9 rat retina. NGF immunoreactivity increases in plexiform layers and inner segments (Magnification x 400).

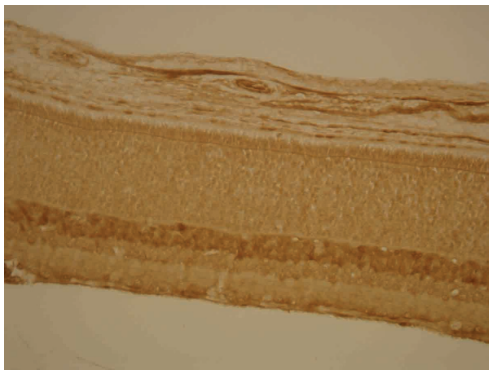

Figure 4. Expression of NGF in P11 rat retina. The immunoreactivity in body's of bipolar, horizontal cells can be seen in the external part of INL (Magnification x 400).

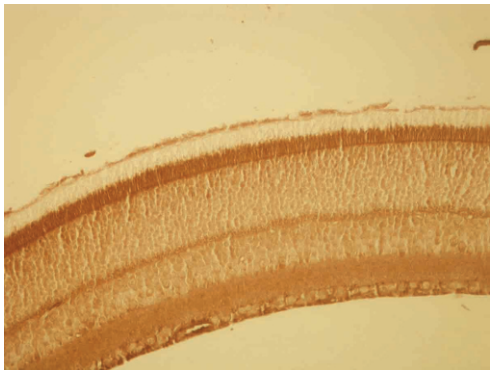

Figure 5. Expression of NGF in P30 rat retina. NGF immunoreactivity in RGL, IPL, OPL and IS are well visible (Magnification x 400).

Discussion

Programmed cell death occurs naturally, as a physiological process, during the embryonic development of multicellular organism. In retina, which belongs to the central nervous system, at least two phases of cell death have been reported to occur during development. An early phase takes place concomitant with the processes of neurogenesis, cell migration and cell differentiation. A later phase affecting mainly neurons occurs when connections are established and synapses

are formed, resulting in selective elimination of inappropriate connections. NGF controls the prog-rammed cell death though binding to the p75 receptor which affects early postmitotic neuroblasts during development. On the other hand, retinal cells may survive the apoptotic effect of NGF by expression the high-affinity TrkA receptor, which switches the pro-appoptotic signaling of P75 into a neurotrophic one [2, 3, 4].

The differention of rat retina is considered with physiological death of retina cells in postnatal development. The normal RGC death period in rat extends from around birth to 6 days later and is especially frequent between postnatal days 1 to 4. The presence of degenerating bipolar cells, amacrine cells and rod has been described at P4 in rat retinas. AC death does not cease until P26 and BC degeneration continues for 48 days. With respect to photoreceptors cells death, a second phase of apoptosis was reported between days 12 and 72, peaking at P23 [5, 6, 7].

We observed the expression of NGF in layered RGC on P1, which increased in the next days. The GCL was composed of layers at P0 and P5, but its thickness decreased and it becomes a monolayer by P17. This suggested a role of NGF in processes of regulation of number of RGC. The stratified RGC in chick retina themselves control their number by secreting NGF, which kills the incoming migratory RGC via p75 on their surface. RGC number control is different between mouse and chick retina. P75 and TrkA seem to be involved in regulation of mouse RGC number in early phase of retinal development, but they may be later adjusted by other molecules [8, 4].

The early positive reaction was found in ventricular layer, from which the photoreceptors (FC), horizontal (HC), bipolar (BC) and amacrine (AC) cells begin their differentiation. In mature rat retina the external parts of INL were positive and the body of BC cells was well visible. Karisson M et all [9, 10] found that NGF plays a role in horizontal cell plasticity and in the survival of horizontal cells (in autocrine mode of action) in avian retina but doesn't take part in survival of AC. We observed the decreasing of expression of AC and well positive BC in rat retina. NGF takes part in differention of BC in rat retina.

Very interesting fact was the early expression in the inner segment of photoreceptors, accordantly their differention and formation. NGF modulates secondary trophic factor expression in Müller cells that may contribute to protection of photoreceptors or increase their apoptosis [11].

Our investigation of expression in IPL and OPL suggested the role of NGF in synaptic plasticity and neurite outgrowth. It would be beneficial this NGF in the rat retina to go under some further investigations because it takes part in degenerating processes [11, 12] but intravitreal injection of endogenous NGF van protect retinal cells from degeneration in experimental retinal detachment [13].

Conclusion

The distribution of NGF in postnatal rat retina suggests that it plays role in neurite outgrowth, regulation of number of ganglion cells and synaptogenesis in inner retina. NGF takes part in differentiation of inner segments of photoreceptor cells.

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