ELSEVIER

available at www.sciencedirect.com

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Short Communication

Neurensin-1 expression in the mouse retina during postnatal development and in cultured retinal neurons

Kizashi Nagata[a,b], *Haruno Suzuki*[a], *Akiko Niiya-Kato*[c], *Shigeru Kinoshita*[c], *Shigeru Taketani*[d], *Masasuke Araki*[a,*]

[a]*Developmental Neurobiology Laboratory, Department of Biological Sciences, Nara Women's University, Nara 630-8506, Japan*
[b]*Department of Biology, Osaka City University, Graduate School, Osaka 558-8585, Japan*
[c]*Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto 602-0841, Japan*
[d]*Department of Biotechnology, Kyoto Institute of Technology, Kyoto 606-8585, Japan*

ARTICLE INFO

Article history:
Accepted 28 December 2005
Available online 28 February 2006

Keywords:
Neurensin-1
Neural retina
Development
Cell culture
Immunocytochemistry
In situ hybridization

ABSTRACT

Neurensin-1/Neuro-p24 (previously named Neuro-p24) is a neuron-specific membrane protein that is localized particularly in neurites. Neurensin-1 is considered to play an essential role in neurite extension during nervous development, regeneration and plasticity. To understand what role Neurensin-1 plays in retinal differentiation, we examined Neurensin-1 distribution and gene expression pattern in the postnatally developing retina of the mouse, because the retina is an excellent model for nervous development. In the postnatal day (PD) 1 retina, intense Neurensin-1 immunoreactivity was found in the optic nerve fiber layer. Faint staining was seen in the ganglion cells, presumptive amacrine and horizontal cells. As the postnatal development proceeded, the optic fibers became more intensely stained in addition to other parts of the retina such as the ganglion cells, inner plexiform layer and horizontal cells. In PD 10 retinas, the horizontal cell processes showed a prominently stained configuration. As the retina developed further to attain maturity, the staining in the retina became less pronounced, although the optic nerves remained positively stained. The distribution of Neurensin-1 mRNA was consistent with these results and confirmed that the ganglion, amacrine and horizontal cells actively synthesize Neurensin-1 in the developing retina. In the retinal cell culture from newborn mice, two types of neural cells were stained for Neurensin-1, one of which showed long processes and appeared presumptive ganglion cells. These results suggest that Neurensin-1 plays a role in the fiber extension of the retinal neurons, as has been observed in other central nervous tissues, and indicate that the developing retina is a suitable experimental model for the analysis of Neurensin function, both in vivo and in vitro.

© 2006 Elsevier B.V. All rights reserved.

Neurons vigorously extend neurites during nervous tissue development and such process extension is a marked and characteristic neuronal feature that is also observed in axonal regeneration and dendritic plasticity in adulthood. Although numerous studies have revealed that several classes of proteins are involved, the precise mechanism for nerve fiber extension needs to be explored in more detail (Brady, 1991; Scott and Luo, 2001; Wong and Ghosh, 2002).

* *Corresponding author*. Fax: +81 742 20 3411.
E-mail address: masaaraki@cc.nara-wu.ac.jp (M. Araki).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.12.131

We previously isolated a novel gene, Neurensin-1/Neuro-p24, which encodes a membrane protein with a molecular mass of 24 kDa (Kadota et al., 1997). Based on its molecular structure and regional distribution profiles in the brain and cultured neurons, Neurensin-1 is specifically localized in growing neurites but not in synaptic vesicles, and we proposed that Neurensin-1 plays certain roles in the transport of small vesicles to the growing distal end of neurites in association with microtubules, thereby contributing to neurite extension (Araki et al., 2002; Ida et al., 2004). COS-7 epithelial cells extended long branching processes when they were transfected with Neurensin-1 cDNA, and Neurensin-1-containing small vesicles are often fused with the plasma membrane at the growth cones. The cell configuration altered depending on the domains deleted; for instance, in a deletion mutant lacking the C-terminal domain, containing a presumably organelle-targeting signal, Neurensin-1 immunoreactive vesicles stayed at the perinuclear region (probably the Golgi region), indicating that the mutated Neurensin-1 protein was not processed at the Golgi region to exit towards organelles. In cultured neuroblastoma neuro2a cells, Neurensin-1 gene was up-regulated when cells were induced to differentiate to neurons with retinoic acid, and immunoreactive vesicles were concentrated at the growth cones. When cells were transfected with antisense oligonucleotide to Neurensin-1, they transiently retracted neurites. Furthermore, in vivo and in vitro pull-down assay confirmed Neurensin-1 binding to tubulin (Ida et al., 2004). These results strongly suggest that Neurensin-1 plays an essential role in neurite extension.

The neural retina is originally derived from the brain vesicle and its developmental, structural and physiological aspects are similar to the central nervous tissue (CNS). The neural retina, therefore, has been considered an excellent experimental model for the study of the CNS. This is also the case with axonal regeneration of the retina (Aguayo, 1985). The developing retina has been intensively studied to analyze the substantial aspects of neural development such as cell proliferation, migration, differentiation and lineage (Adler, 2000; Nguyen and Arnheiter, 2000). Neurite extension is a crucial aspect of neural differentiation of retinal neurons during regeneration and development. During retinal development, retinal precursor cells proliferate, migrate to the appropriate position and generate various types of retinal cells according to a genetically programmed timetable; in the rodent retina, the majority of ganglion and horizontal cells appear earlier, while rods and bipolar cells are produced later (Hinds and Hinds, 1979; Shaw and Weber, 1983; Young, 1985). Immediately after cells are born, they initiate neurite growth by activating a set of genes necessary for the synthesis and transport of membrane vesicles (Gordon-Weeks, 2000; Skene, 1989). Neurensin-1 must be an important gene that is up-regulated for such a process, as suggested by previous studies. In the present study, we analyzed the localization of Neurensin-1 proteins and mRNA during the postnatal development of the mouse retina to investigate the role of this interesting gene in retinal development. We also examined Neurensin-1 expression in cultured retinal cells of the neonate mouse to determine whether it is correlated with the in vivo developing retina. The results suggest that Neurensin-1 plays an important role in the axonal growth of retinal neurons, particularly in the ganglion and horizontal cells.

Localization of Neurensin-1 immunoreactivity in the postnatal developing mouse retina. Intense Neurensin-1 immunoreactivity was observed in the optic nerve fibers at the early stage of postnatal development (Fig. 1). This staining in the optic nerve fiber layer became faint as development proceeded and disappeared in the mature retina. The optic nerve, however, remained positively stained in later development (Fig. 1F). The ganglion cell bodies were faintly stained and some of the cells located at the innermost position of the neuroblastic layer (presumably amacrine cells) were also stained lightly on PD1 and 4 (Figs. 1A, B). Discrete cell bodies in the outer position of the neuroblastic cell layer were found to be positively stained on PD1 (Fig. 1A). This staining was faint on PD1 but was obviously recognized on PD4. These cells were identified as differentiating horizontal cells from their position and morphology (Chien and Liem, 1995). The inner plexiform layer was also lightly stained. On PD10, cell bodies of presumptive horizontal cells were not stained but many fibers were still positively stained in the outer plexiform layer (Fig. 1C). As the retina developed further, immunostained fibers in the outer plexiform layer gradually disappeared and no staining could be seen on PD20, when only faint staining was seen in the nerve fiber layer, but the optic nerves outside the retina were still positively stained. No reaction product was found in the outer nuclear layer (rod cell layer).

Localization of Neurensin-1 mRNA in the developing mouse retina. To identify the cell bodies that synthesize Neurensin-1, we performed in situ hybridization with developing mouse retinas (Fig. 2). On PD5, the ganglion cells were intensely stained and presumptive amacrine cells at the innermost position of the neuroblast layer were also stained (Fig. 2A). Cell bodies located at the outer portion of the neuroblast layer were also stained. These cells were considered horizontal cells and they still extended several fine neurites towards the inner layer. The whole profile of staining did not change on PD10 (Fig. 2D). On PD15, ganglion cells as well as amacrine cells were still stained, but a little less intensely than was observed in the previous stages. The horizontal cells were not stained anymore. These staining profiles and the temporal changes during postnatal development were roughly correlated with those of immunocytochemical staining. RT-PCR also revealed Neurensin-1 gene expression in the postnatal retinas as a single band at a position corresponding to the mRNA size (data not shown).

Localization of Neurensin-1 in cultured mouse retina. To investigate the molecular function of Neurensin-1 in retinal development in our further study, it is essential to examine whether cultured mouse retinal cells also express the Neurensin-1 gene, so we performed a cell culture study of newborn mouse retinas. When cultured under the standard condition, cells formed cell aggregates on an epithelial sheet and Neurensin-1 positive processes were found within the aggregates. These stained processes were usually short and stayed within the aggregates (Figs. 3A, B) and gradually

Download English Version:

https://daneshyari.com/en/article/4332928

Download Persian Version:

https://daneshyari.com/article/4332928

Daneshyari.com