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Expression of brain-derived neurotrophic factor in cholinergic and dopaminergic amacrine cells in the rat retina and the effects of constant light rearing

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Abstract

Brain-derived neurotrophic factor (BDNF) regulates many aspects of neuronal development, including survival, axonal and dendritic growth and synapse formation. Despite recent advances in our understanding of the functional significance of BDNF in retinal development, the retinal cell types expressing BDNF remains poorly defined. The goal of the present study was to determine the localization of BDNF in the mammalian retina, with special focus on the subtypes of amacrine cells, and to characterize, at the cellular level, the effects of constant light exposure during early postnatal period on retinal expression of BDNF. Retinas from 3-week-old rats reared in a normal light cycle or constant light were subjected to double immunofluorescence staining using antibodies to BDNF and retinal cell markers. BDNF immunoreactivity was localized to ganglion cells, cholinergic amacrine cells and dopaminergic amacrine cells, but not to AII amacrine cells regardless of rearing conditions. Approximately 75% of BDNF-positive cells in the inner nuclear layer were cholinergic amacrine cells in animals reared in a normal lighting condition. While BDNF immunoreactivity in ganglion cells and cholinergic amacrine cells was significantly increased by constant light rearing, which in dopaminergic amacrine cells was apparently unaltered. The overall structure of the retina and the density of ganglion cells, cholinergic amacrine cells and AII amacrine cells were unaffected by rearing conditions, whereas the density of dopaminergic amacrine cells was significantly increased by constant light rearing. The present results indicate that cholinergic amacrine cells are the primary source of BDNF in the inner nuclear layer of the rat retina and provide the first evidence that cholinergic amacrine cells may be involved in the visual activity-dependent regulation of retinal development through the production of BDNF. The present data also suggest that the production or survival of dopaminergic amacrine cells is regulated by early visual experience.

Keywords: brain-derived neurotrophic factor; retina; amacrine cell; cholinergic; dopaminergic; constant light; development; immunohistochemistry

1. Introduction

Brain-derived neurotrophic factor (BDNF) has been shown to regulate many aspects of neuronal development, including survival, axonal and dendritic growth and synapse formation (Bibel and Barde, 2000). In the retina, BDNF influences the morphological maturation of ganglion cells (Alsina et al., 2001; Cohen-Cory and Fraser, 1995; Landi et al., 2007; Lom et al., 2002) and the morphological and neurochemical phenotypes of amacrine cells (Cellerino et al., 1998; Cellerino et al., 1999, 2003; Rickman and Bowes Rickman, 1996). In addition, BDNF can support the survival of axotomized retinal ganglion cells (Mansour-Robaey et al., 1994; Mey and Thanos, 1993) and protect photoreceptors from the damaging effects of constant light (LaVail et al., 1992) or from inherited retinal degeneration (LaVail et al., 1998).

It has been shown that the expression of BDNF in the retina is affected by early visual experience. BDNF expression in the retina is enhanced by constant light rearing (Pollock et al., 2001) and reduced by constant dark rearing (Landi et al., 2007; Pollock et al., 2001) or monocular deprivation (Mandolesi et al., 2005; Seki et al., 2003). Recent evidence has further

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indicated that alterations in retinal BDNF levels induced by early visual experience can influence retinal neurochemical development (Lee et al., 2006) and visual cortical connections (Mandolesi et al., 2005).

Despite recent advances in our understanding of the functional significance of BDNF in the retina, the retinal cell types expressing BDNF remains poorly defined. Previous immunohistochemical and in situ hybridization studies have demonstrated that retinal ganglion cells and a population of cells in the inner nuclear layer (INL) express BDNF (Cohen-Cory et al., 1996; Perez and Caminos, 1995; Seki et al., 2003; Vecino et al., 1998). Although the BDNF-expressing cells in the INL are most likely amacrine cells (Perez and Caminos, 1995; Seki et al., 2003), the identity of amacrine cell subtypes expressing BDNF remains unknown. Considering the morphological, neurochemical and functional diversity of amacrine cells (Vaney, 1990), determining the amacrine cell subtypes expressing BDNF would provide crucial information about the function and mechanism of action of BDNF in the retina. Therefore, we investigated the cellular localization of BDNF in the rat retina, with special focus on the subtypes of amacrine cells. As the BDNF content in the rat retina has been shown to increase by constant light rearing (Pollock et al., 2001), the effects of constant light exposure during early postnatal period on the expression of retinal BDNF was also investigated at the cellular level.

2. Materials and methods

2.1. Animals and tissue preparation

Male and female Wistar rats (Charles River Laboratories Japan) were born and reared either in a normal light cycle (12:12 h light/dark cycle: LD) or constant light (LL). Light intensity at the level of the cages was approximately 200 lux. Animals were killed by decapitation after anesthesia with diethyl ether on postnatal day 21 (P21) ($n = 10$ for each rearing condition). Normally reared animals were killed in the middle of the light phase and LL animals were killed at the same time of day. The eyecups with the cornea and lens removed were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 3 hrs, rinsed in 15% and 30% sucrose in phosphate buffer, and frozen with dry ice–isopentane. Cryostat sections were cut at 10 μm through the optic disc along the dorsoventral axis and collected on APS-coated glass slides (Superfrost; Matsunami Glass, Osaka, Japan). For whole-mount preparations, the retinas were dissected free from the choroid and sclera following fixation, rinsed in sucrose solutions, and subjected to three freeze-thaw cycles to improve antibody penetration. The retinas being compared (LD vs. LL) were always processed in parallel. All experimental procedures were conducted in accordance with a research protocol approved by our institutional ethical review committee of animal experiments.

2.2. Antibodies

Rabbit polyclonal antibody raised against a peptide corresponding to the N-terminus of human BDNF (Santa Cruz Biotechnology, Santa Cruz, USA) was used in the present study. This antibody recognizes both precursor and mature BDNF (according to the manufacturer) and has been shown to specifically label BDNF in the rat retina (Seki et al., 2003). Mouse monoclonal antibodies to Brn3a (Santa Cruz Biotechnology), tyrosine hydroxylase (TH) (Sigma, St. Louis, MO, USA) and parvalbumin (Sigma), and goat polyclonal antibody to choline acetyltransferase (ChAT) (Chemicon International, Temecula, CA, USA) were used to identify retinal cell types. Alexa Fluor 488 donkey anti-rabbit IgG (Molecular Probes, Eugene, OR, USA), Alexa Fluor 594 donkey anti-rabbit IgG (Molecular Probes), Alexa Fluor 488 donkey anti-mouse IgG (Molecular Probes), and Alexa Fluor 568 donkey anti-goat IgG (Molecular Probes) were used as secondary antibodies.

2.3. Immunohistochemistry

For BDNF immunostaining, sections were sequentially incubated with 10% normal donkey serum for 30 min, anti-BDNF antibody overnight, and Alexa Fluor 594 donkey anti-rabbit IgG for 30 min. Immunohistochemical controls were performed by replacing primary antibody with normal rabbit serum or by omission of primary antibody. No specific signals were detected in control sections (data not shown). Double immunolabeling was performed using antibodies to retinal cell markers in combination with anti-BDNF. Sections were incubated in a mixture of primary antibodies overnight, followed by a mixture of secondary antibodies for 30 min. Dilutions of primary antibodies were: BDNF 1:2000, Brn3a 1:50, ChAT 1:400, TH 1:2000, and parvalbumin 1:5000. All secondary antibodies were diluted 1:1000. All dilutions were performed in 0.1 M phosphate-buffered saline (PBS, pH 7.4) containing 0.3% Triton X-100 and 1% bovine serum albumin. Double immunostaining for BDNF and TH was also conducted with the whole-mount retinas. The whole-mount tissue was incubated in a mixture of primary antibodies for two overnight periods, followed by a mixture of secondary antibodies overnight. For whole-mount staining, dilutions were performed in PBS containing 0.5% Triton X-100, 1% bovine serum albumin, and 0.05% sodium azide. Fluorescence signals were examined and images obtained by confocal laser scanning microscope (LSM510 META; Carl Zeiss, Germany). Retinas being compared (LD vs. LL) were photographed using the same settings of confocal imaging.

2.4. Quantitation and statistics

The thickness of the outer nuclear layer (ONL), INL, and inner plexiform layer (IPL) visualized by differential interference contrast (DIC) optics was measured in 6 fields (232 μm × 232 μm each) per sample ($n = 4$) in the central region of retinal sections, defined as a 1 mm region from the optic disc margin towards the periphery. The density of Brn3a-positive ganglion cells and BDNF-positive, ChAT-positive and parvalbumin-positive amacrine cells was quantitated in 12 fields (328 μm × 328 μm each) per sample ($n = 4$) in the central