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BRAIN-DERIVED NEUROTROPHIC FACTOR INHIBITS OSMOTIC SWELLING OF RAT RETINAL GLIAL (MÜLLER) AND BIPOLAR CELLS BY ACTIVATION OF BASIC FIBROBLAST GROWTH FACTOR SIGNALING

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Abstract—Water accumulation in retinal glial (Müller) and neuronal cells resulting in cellular swelling contributes to the development of retinal edema and neurodegeneration. Intravitreal administration of neurotrophins such as brain-derived neurotrophic factor (BDNF) is known to promote survival of retinal neurons. Here, we show that exogenous BDNF inhibits the osmotic swelling of Müller cell somata induced by superfusion of rat retinal slices or freshly isolated cells with a hypoosmotic solution containing barium ions. BDNF also inhibited the osmotic swelling of bipolar cell somata in retinal slices, but failed to inhibit the osmotic soma swelling of freshly isolated bipolar cells. The inhibitory effect of BDNF on Müller cell swelling was mediated by activation of tropomyosin-related kinase B (TrkB) and transactivation of fibroblast growth factor receptors. Exogenous basic fibroblast growth factor (bFGF) fully inhibited the osmotic swelling of Müller cell somata while it partially inhibited the osmotic swelling of bipolar cell somata. Isolated Müller cells displayed immunoreactivity of truncated TrkB, but not full-length TrkB. Isolated rod bipolar cells displayed immunoreactivities of both TrkB isoforms. Data suggest that the neuroprotective effect of exogenous BDNF in the retina is in part mediated by prevention of the cytotoxic swelling of retinal glial and bipolar cells. While BDNF directly acts on Müller cells by activation of TrkB, BDNF indirectly acts on bipolar cells by inducing glial release of factors like bFGF that inhibit bipolar cell swelling.

Key words: brain-derived neurotrophic factor, osmotic stress, cell swelling, glia, bipolar cell, retina.

INTRODUCTION

Blinding retinal diseases such as age-related macular degeneration, retinitis pigmentosa, and glaucoma are characterized by degeneration of photoreceptors and/or inner retinal neurons. Intravitreal administration of various growth factors, cytokines, and neurotrophins including brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), nerve growth factor (NGF), and basic fibroblast growth factor (bFGF) was shown to promote the survival of photoreceptors and retinal neurons under pathological conditions (Faktorovich et al., 1992; LaVail et al., 1992; Unoki and LaVail, 1994; Mansour-Robaey et al., 1994; Harada et al., 2000; Hauck et al., 2006; reviewed in Bringmann et al., 2009). The neurotrophic rescue of photoreceptors and neurons is, at least in part, indirectly mediated by regulation of the trophic factor production in retinal glial (Müller) cells (Wexler et al., 1998; Wahlin et al., 2000; Harada et al., 2002; Saito et al., 2009). Müller cells are known to produce various neuroprotective factors including BDNF, bFGF, NGF, and GDNF (Harada et al., 2000, 2002; Seki et al., 2005; Hauck et al., 2006; Garcia et al., 2014). In addition to NGF and GDNF, BDNF stimulates the production of bFGF in Müller cells (Harada et al., 2000, 2002; Hauck et al., 2006).

Neurotrophins control the neuronal survival via two types of receptors: the tropomyosin-related kinase (Trk) family of high-affinity tyrosine kinase receptors predominantly transmits prosurvival signals, while the low-affinity p75 neurotrophin receptor ($p75^{NTR}$) mainly transmits anti-survival signals (Casaccia-Bonnefil et al., 1999; Blum and Konnerth, 2005). In the murine

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Abbreviations: BDNF, brain-derived neurotrophic factor; bFGF, basic fibroblast growth factor; GDNF, glial cell line-derived neurotrophic factor; HEPES, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; K252a, (9*S*,10*R*,12*R*)-2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-9,12-epoxy-1*H*-diindolo[1,2,3-*fg*:3′,2′,1′-*kl*]pyrrolo[3,4-*i*][1,6]benzodiazocine-10-carboxylic acid methyl ester; LSM, laser scanning microscope; LY341495, (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid; NGF, nerve growth factor; $p75^{NTR}$, p75 neurotrophin receptor; PD173074, N-[2-[[4-(diethylamino) butyl]amino-6-(3,5-dimethoxyphenyl)pyrido[2,3-d]pyrimidin-7-yl]-N′-(1,1-dimethylethyl)urea; PKCα, protein kinase Cα; SB431542, 4-(5-benzol[1,3]dioxol-5-yl-4-pyridin-2-yl-1H-imidazol-2-yl)-benzamide hydrate; SU1498, (E)-3-(3,5-diisopropyl-4-hydroxyphenyl)-2-[(3-phenyl-n-propyl)amino-carbonyl]acrylnitrile; SU5402, 2-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)methyl]-4-methyl-1H-pyrrole-3-propanoic acid; TGF, transforming growth factor; TrkB, tropomyosin-related kinase B; VEGF, vascular endothelial growth factor.

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neuroretina, several cell types such as Müller, amacrine, and retinal ganglion cells express the tropomyosin-related kinase B (TrkB) receptor for BDNF, whereas photoreceptors do not (Rohrer et al., 1999; Wahlin et al., 2000; Harada et al., 2011; Shen et al., 2012). Likewise, BDNF cannot exert its effects directly on most photoreceptors of the rat retina because they do not express BDNF receptors (Suzuki et al., 1998; Wahlin et al., 2001; Asai et al., 2007). TrkB is expressed by green–red cones (Di Polo et al., 2000; Grishanin et al., 2008) which represent less than 1% of all photoreceptors of the rat retina (Szél and Röhlich, 1992). Activation of $p75^{NTR}$ was suggested to have either pro- or antisurvival effects in the retina, in dependence on the experimental model studied (Wexler et al., 1998; Harada et al., 2000, 2002). NGF acting at $p75^{NTR}$ was suggested to decrease the production of bFGF by Müller cells resulting in increased photoreceptor apoptosis (Harada et al., 2000). In another study, BDNF was suggested to promote the survival of bipolar cells through activation of $p75^{NTR}$ on Müller cells and subsequent secretion of bFGF from Müller cells which directly rescues bipolar cells (Wexler et al., 1998).

Development of tissue edema is a major complication of ischemic–hypoxic and inflammatory retinal diseases including exudative age-related macular degeneration, diabetic retinopathy, uveitis, and artherosclerotic vascular retinal disorders. In uveitis and diabetic retinopathy, retinal edema is the major cause of visual deterioration (Bresnick, 1983; Rothova et al., 1996). In addition to the breakdown of the blood-retinal barrier (vasogenic edema), osmotic swelling of retinal neurons and glial cells (cytotoxic edema) may contribute to the development of edema and neurodegeneration in the retina (Bringmann et al., 2005). Water accumulation in Müller glial cells was found in various animal models of retinal ischemia-hypoxia and diabetic retinopathy (Stepinac et al., 2005; Kaur et al., 2007; Kumar et al., 2013).

Hypoosmolarity of the extracellular fluid is a characteristic of intense neuronal activity in the retina (Dmitriev et al., 1999). Normally, Müller cells do not swell in hypoosmotic stress (Pannicke et al., 2004), suggesting that they possess endogenous volume-regulatory mechanisms that prevent osmotic cell swelling. Müller cell volume regulation was shown to depend on the passive transmembrane potassium flux (Pannicke et al., 2004) and activation of an endogenous glutamatergic–purinergic signaling cascade (Uckermann et al., 2006; Wurm et al., 2008). Various neuroprotective factors including vascular endothelial growth factor (VEGF), erythropoietin, osteopontin, and NGF activate this signaling cascade (Wurm et al., 2008; Krügel et al., 2010; Wahl et al., 2013; Garcia et al., 2014), suggesting that inhibition of cytotoxic Müller cell swelling might represent one neuroprotective mechanism. Because water accumulation in retinal glial and neuronal cells is a pathogenic factor involved in retinal degeneration under ischemic–hypoxic and inflammatory conditions (Bringmann et al., 2005), we determined whether BDNF influences the osmotic swelling of rat Müller and bipolar cells.

EXPERIMENTAL PROCEDURES

Materials

Mitotracker Orange was obtained from Life Technologies (Darmstadt, Germany). Cyclotraxin-B, (9*S*,10*R*,12*R*)-2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-9,12-epoxy-1*H*-diindolo[1,2,3-*fg*:3′,2′,1′-*kl*]pyrrolo[3,4-*i*][1,6]benzodiazocine-10-carboxylic acid methyl ester (K252a), (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid (LY341495), MRS2179, N-[2-[[4-(diethylamino)butyl]amino-6-(3,5-dimethoxyphenyl)pyrido[2,3-d]pyrimidin-7-yl]-N′-(1,1-dimethylethyl)urea (PD173074), staurosporine, 2-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)methyl]-4-methyl-1H-pyrrole-3-propanoic acid (SU 5402), and recombinant human BDNF (2837) were from Tocris Bioscience (Wiesbaden-Nordenstadt, Germany). Recombinant human bFGF (GF003-AF) and TAT-conjugated Pep5 (506181) were purchased from Merck Millipore (Darmstadt, Germany). Recombinant human hemoglobin (H7379), 4-(5-benzol[1,3]dioxol-5-yl-4-pyridin-2-yl-1H-imidazol-2-yl)-benzamide hydrate (SB43 1542), and all other agents used were purchased from Sigma-Aldrich (Taufkirchen, Germany), unless stated otherwise. The following antibodies were used: rabbit anti-BDNF (detects both proBDNF and mature BDNF; sc-546; 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-TrkB gp95 (sc-119; 1:200; Santa Cruz), rabbit anti-TrkB gp145 (sc-12; 1:200; Santa Cruz), mouse anti-glutamine synthetase (1:200; Merck Millipore), mouse anti-protein kinase Cα (PKCα; 1:200; Santa Cruz), Cy3-coupled goat anti-rabbit IgG (1:400; Jackson Immuno Research, Newmarket, UK), and Cy2-coupled goat anti-mouse IgG (1:200; Jackson Immuno Research).

Animals

All experiments were done in accordance with the European Communities Council Directive 86/609/EEC, and were approved by the local authorities (University of Leipzig Medical Faculty and Landesdirektion Leipzig). Adult Long-Evans rats (250–350 g; both sexes) were bred in the Medical-Experimental Center of the University of Leipzig Medical Faculty. Animals were killed with carbon dioxide, and the retinas were removed.

Cell soma recording

Retinal slices and suspensions of dissociated retinal cells were prepared as described (Wurm et al., 2008). Müller and bipolar cell somata in retinal slices were identified as recently described (Garcia et al., 2014). All experiments were performed at room temperature (20–23 °C). Retinal slices or isolated cells were transferred to a custom-made perfusion chamber and kept submerged in extracellular solution. Slices or cells were loaded with the vital dye Mitotracker Orange (1 μm) for 3 min. Recordings were made with a confocal laser scanning microscope (LSM 510 Meta; Zeiss, Oberkochen, Germany) and an Achroplan 63×/0.9 water immersion objective (Zeiss). The pinhole was set at 151 μm; the