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OSTEOPONTIN INHIBITS OSMOTIC SWELLING OF RETINAL GLIAL (MÜLLER) CELLS BY INDUCING RELEASE OF VEGF

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Abstract—Osmotic swelling of retinal neurons and glial cells is an important pathogenic factor of retinal edema formation. Here, we show that the neuroprotective factor osteopontin (OPN), which is released from retinal glial (Müller) cells after stimulation of the cells with glial cell line-derived neurotrophic factor (Del Río et al., 2011, Glia 59:821–832), inhibits the swelling of rat Müller cells induced by hypoosmotic exposure of retinal slices in the presence of barium ions and H_2O_2, respectively, and in slices of postischemic retinas. OPN did not inhibit the hypoosmotic swelling of bipolar cells in slices of control and postischemic retinas. The inhibitory effect of OPN on Müller cell swelling was dose-dependent, with a half-maximal effect at ~0.6 ng/ml. The effect of OPN was abrogated in the presence of pharmacological blockers of vascular endothelial growth factor (VEGF) receptor-2, metabotropic glutamate receptors, and purinergic receptors ($P2Y_1$, adenosine A_1 receptors), as well as of a neutralizing anti-VEGF antibody. The data suggest that OPN induces the release of VEGF, glutamate, ATP, and adenosine from Müller cells. The effect of OPN was also prevented by blockers of voltage-gated sodium channels (tetrodotoxin), T-type voltage-gated calcium channels (kurtoxin), potassium channels (clofilium), and chloride channels 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB). The swelling-inhibitory effect of OPN was dependent on intracellular calcium signaling, activation of phospholipase C and protein kinase C, and vesicular exocytosis of glutamate. In retinal slices, Müller glial cells display immunoreactivity of OPN. The data suggest that Müller cell-derived OPN has (in addition to the effects on photoreceptors and retinal neurons) autocrine effects. The neuroprotective effects of OPN may be in part mediated by the prevention of cytotoxic Müller cell swelling and the release of VEGF and adenosine from Müller cells.

Key words: osteopontin, VEGF, osmotic swelling, ischemia, glia, retina.

INTRODUCTION

Retinal diseases such as age-related macular degeneration, retinitis pigmentosa, and glaucoma are characterized by a degeneration of photoreceptors and/or inner retinal neurons. Various neurotrophic and growth factors, or combinations of the factors, promote the survival of photoreceptors and retinal neurons. Brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor, basic fibroblast growth factor (bFGF), and other factors rescue photoreceptors and retinal neurons such as retinal ganglion and bipolar cells from apoptosis under distinct conditions (reviewed in Bringmann et al., 2009). However, the mechanisms of the neuroprotective action of the factors are not fully understood. It has been proposed that part of the neurotrophic rescue of photoreceptor and retinal neurons is indirect, mediated by the interaction of neurotrophic factors with retinal glial (Müller) cells that in turn release trophic factors that act directly on photoreceptor and neuronal cells (Wen et al., 1995; Wexler et al., 1998; Harada et al., 2000; Wahlin et al., 2000; Garcia and Vecino, 2003). One factor which is proposed to protect photoreceptors indirectly via the activation of Müller cells is the glial cell line-derived neurotrophic factor (GDNF) (Hauck et al., 2006). GDNF protects retinal neurons from excitotoxicity by inducing upregulation of glutamate transporters in retinal glial cells (Koeberle and Bähr, 2008). GDNF induces the expression of BDNF and bFGF in Müller cells (Harada et al., 2003). GDNF was also shown to induce expression and secretion of osteopontin (OPN) from murine Müller cells (Del Río et al., 2011). OPN itself

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Abbreviations: BAPTA-AM, bis-(o-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid acetoxymethyl ester; BDNF, brain-derived neurotrophic factor; bFGF, basic fibroblast growth factor; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; GDNF, glial cell line-derived neurotrophic factor; Gö6976, 12-(2-cyanoethyl)-6,7,12,13-tetrahydro-13-methyl-5-oxo-5H-indolo(2,3-a)pyrrolo(3,4-c)-carbazole; HEPES, hydroxyethyl piperazineethanesulfonic acid; LY341495, (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid; MRS2179, N^6-methyl-2′-deoxyadenosine-3′,5′-bisphosphate; NPPB, 5-nitro-2-(3-phenylpropylamino)benzoic acid; OPN, osteopontin; SNAP, *S*-nitroso-*N*-acetyl-penicillamine; SU1498, (E)-3-(3,5-diisopropyl-4-hydroxyphenyl)-2-[(3-phenyl-*n*-propyl)amino-carbonyl]acrylnitrile; U73122, 1-[6-[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1H-pyrrole-2,5-dione; VEGF, vascular endothelial growth factor.

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exerts a pro-survival effect in cultured photoreceptor cells and reduces the level of apoptosis in retinal explants from retinal degeneration-1 mice (Del Río et al., 2011). Retinal OPN is upregulated in experimental glaucoma and protects retinal ganglion cells from death (Birke et al., 2010). Thus, OPN represents a candidate molecule which mediates the neuroprotective action of Müller cells. However, it is not known whether OPN also exerts autocrine effects on Müller cells.

The development of tissue edema is a complication of ischemic–hypoxic and inflammatory retinal diseases including diabetic retinopathy, uveitis, and artherosclerotic vascular disorders. In uveitis and diabetic retinopathy, retinal edema is the major cause of visual deterioration (Bresnick, 1983; Rothova et al., 1996). By compression of retinal neurons, nerve fibers, and blood vessels, edema contributes to ischemic–hypoxic conditions, functional impairment of photoreceptors, and death of retinal neurons. In addition to the breakdown of the blood–retinal barrier (vasogenic edema) and the dysregulation of the fluid clearance across retinal glial and pigment epithelial cells, osmotic swelling of retinal neurons and glial cells (cytotoxic edema) is one pathogenic factor involved in the development of retinal edema (Bringmann et al., 2004, 2005). Müller cells possess an endogenous glutamatergic–purinergic signaling mechanism which prevents cellular swelling under hypoosmotic conditions (Uckermann et al., 2006; Wurm et al., 2008, 2010). Hypoosmolarity of the extracellular space fluid is a characteristic feature of intense neuronal activity in the normal and ischemic retina (Dmitriev et al., 1999). It has been shown that the neuroprotective factor erythropoietin (Grimm et al., 2002) activates the swelling-inhibitory signaling cascade by inducing the release of vascular endothelial growth factor (VEGF) from Müller cells; VEGF induces the release of glutamate and, subsequently, adenosine 5′-triphosphate (ATP) and adenosine from the cells (Krügel et al., 2010). The final step of the cascade is the adenosine A_1 receptor-induced opening of potassium and chloride channels in the Müller cell membrane; the ion efflux compensates the osmotic gradient across the plasma membrane and, thus, prevents osmotic swelling of the cells (Skatchkov et al., 2006; Uckermann et al., 2006; Wurm et al., 2008, 2009). In the present study we show that OPN prevents the osmotic swelling of Müller cells (but not bipolar cells) of the rat retina, by triggering the above-described cell volume-regulatory signaling cascade.

EXPERIMENTAL PROCEDURES

Materials

Mitotracker Orange (chloromethyltetramethylrosamine) was obtained from Molecular Probes (Eugene, OR, USA). Papain was from Roche Molecular Biochemicals (Mannheim, Germany). Human recombinant human OPN was purchased from R&D Systems (Wiesbaden, Germany). Human recombinant VEGF-A_{165} was from Chemicon (Temecula, CA, USA). N^6-Methyl-2′-deoxyadenosine-3′,5′-bisphosphate (MRS2179) and (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid (LY341495) were from Tocris Cookson (Ellisville, MO, USA). 12-(2-Cyanoethyl)-6,7,12,13-tetrahydro-13-methyl-5-oxo-5H-indolo(2,3-a)pyrrolo(3,4-c)-carbazole (Gö6976) was from Merck Bioscience (Darmstadt, Germany). All other agents used were purchased from Sigma–Aldrich (Taufkirchen, Germany), unless stated otherwise. The following antibodies were used: neutralizing goat anti-human VEGF-$A_{121/165}$ (1 μg/ml; R&D Systems), goat anti-OPN (1:20; R&D Systems), rabbit anti-α_v (1:200; Millipore), rabbit anti-CD44 (1:200; Sigma–Aldrich), mouse anti-glutamine synthetase (1:1000; GS-6 clone; Chemicon), Cy2-coupled donkey anti-goat (1:400; Jackson Immuno Research, Suffolk, UK), Cy2-coupled goat anti-rabbit (1:400; Jackson), and Cy3-coupled goat anti-mouse (1:400; Jackson).

Animals and experimental retinal ischemia-reperfusion

All experiments were done in accordance with the European Communities Council Directive 86/609/EEC, and were approved by the local authorities (Faculty of Medicine of the University of Leipzig and Landesdirektion Leipzig). All efforts were made to minimize the number of animals used and their suffering. Adult Long-Evans rats (250–350 g) were used. Animals were maintained with free access to water and food in an air-conditioned room on a 12-h light–dark cycle, and were killed with carbon dioxide. In five animals, retinal ischemia-reperfusion was induced in one eye. The animals were anesthesized with intramuscular ketamine (50 mg/kg body weight) and xylazine (3 mg/kg). The anterior chamber of the treated eye was cannulated from the pars plana with a 30-gauge infusion needle, connected to a bag containing normal saline. The intraocular pressure was increased to 160-mm Hg for 60 min by elevating the saline bag. After 3 days, the animals were killed with carbon dioxide, and the eyes were removed.

Preparation of retinal slices

Pieces of freshly isolated retinas (5 × 5 mm) were placed, with the photoreceptor side down, onto membrane filters (mixed cellulose ester, 0.45 μm pore size; Schleicher & Schuell MicroScience, Dassel, Germany). Retinal slices (thickness, 1 mm) were cut from these tissues adhering to the membrane filters using a custom-made cutter equipped with a razor blade.

Cell soma swelling

All experiments were performed at room temperature (20–23 °C). To determine the volume changes of Müller and bipolar cell somata induced by hypoosmotic challenge, the cell bodies in the inner nuclear layer of retinal slices were recorded (Fig. 1A). Whereas Müller cell bodies are localized to the central part of the inner nuclear layer, bipolar cell bodies are localized to the outer part of this layer. In addition, the outer processes