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Endothelin receptors in the detached retina of the pig

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Abstract

Endothelin-1 (ET-1) is a potent vasoconstrictor that causes hypoperfusion of the neurosensory retina. We investigated immunohistochemically the expression of the receptors for ET-1, ET_A and ET_B, in control and locally detached retinas of the pig. Immunoreactivity for ET_A was expressed in the innermost retinal layers and in the outer plexiform layer in control retinas, and was additionally strongly expressed by retinal blood vessels at 7 days after detachment of the sensory retina from the pigment epithelium. Immunoreactivity for ET_B was expressed by the innermost retinal layers, by ganglion cell somata, and by Müller glial cells in the control tissue, and was not altered in its expression after detachment. The vascular expression of ET_A may suggest a hypoperfusion of the retina after detachment.

Keywords: Endothelin; Immunohistochemistry; Blood vessels; Müller cells; Retinal detachment; Pig

Detachment of the neurosensory retina from the pigment epithelium causes complex alterations and remodeling of the retinal tissue. In addition to the deconstruction of outer segments and photoreceptor cell death, there are morphological and biochemical alterations of the inner retinal neurons [5], as well as a fast activation of pigment epithelial and macro- and microglial cells [6,20]. The activation of glial cells in the experimentally detached retina begins within minutes of detachment [6] and develops during the first hours and days after creation of the detachment [20]. The gliosis of Müller cells (the predominant macroglial cell in the retina) is characterized by the upregulation of the expression of immunoreactivity for the intermediate filaments vimentin and glial fibrillary acidic protein, by cellular hypertrophy, and by distinct physiological alterations such as upregulation of the responsiveness upon stimulation of distinct receptors [20].

A variety of growth and activating factors may induce glial cell activation in the injured retina. Upon retinal detachment, mechanical stress may activate glial cells, and intraretinally released growth factors and cytokines activate mitogen-activated protein kinases and transcription factors, as observed previously [6]. Other factors that were proposed to induce glial cell activation upon injury, include the endothelins that bind at endothelin (ET) A and B receptors. In the brain and spinal cord, activation of astrocytes is normally accompanied by upregulation of ET_B receptors [7,21] while it is not known if this is also the case in the sensory retina. ET-1 is a potent vasoconstrictor of retinal blood vessels [1,11], as well as a mitogen for retinal vascular smooth muscle cells and pericytes [8], and has been suggested to be implicated in the pathogenesis of important retinopathies such as glaucoma [10] and diabetic retinopathy [8]. Enhanced action or levels of ET-1 are associated with decreased blood flow in the retinas of diabetic animals. It has been shown that ET-1 promotes gliosis of optic nerve head astrocytes in vitro [13], and that experimental glaucoma or optic nerve transection increases the expression of ET-1 and ET_B in optic nerve astrocytes [12,14]. By using autoradiography, it has been shown that ET_A-like binding sites are expressed by blood vessels of the human retina while ET_B-like binding sites are localized to neural and glial cells of the retina [9]. In other studies,

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the presence of ET_A and ET_B receptors on vascular smooth muscle cells of retinal vessels, and of ET_B receptors within the ganglion cell layer, has been shown [2,17]. In retinas of diabetic rats, an upregulation of endothelins, ET_A and ET_B has been described [3]. However, it is not known whether Müller glial cells in the sensory retina express receptors for endothelins, and whether the expression is altered during retinal injury. Therefore, we investigated the expression of ET_A and ET_B proteins in retinal slices derived from pig eyes. We immunohistochemically stained slices from control retinas, as well as from retinas that were detached from the pigment epithelium for 7 days. As a marker for glial cells, the slices were co-stained against vimentin.

All experiments were carried out in accordance with applicable German laws and with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Three young adult domestic pigs (17–22 kg; both sexes) were used. Twenty-four hours before and after surgery, the food intake of the animals was restricted with free access to water. Intravenous azaperon (15 mg/kg; Cilag-Janssen, Neuss, Germany), atropin (0.2 mg/kg; Braun, Melsungen, Germany) and ketamine (3 mg/kg; Ratiopharm, Ulm, Germany) were administered for premedication. The anesthesia was induced with thiopental (8 mg/kg i.v.; Trapanal, Byk Gulden, Konstanz, Germany) and maintained with isoflurane (Forene, Abbott, Wiesbaden, Germany). Ventilation was performed using the Julian respirator (Draegerwerk AG, Luebeck, Germany) with a F_iO_2 of 40%. Rhegmatogenous detachment was created in one eye per animal; the other eye served as untreated control. The pupils of the eyes were dilated by topical tropicamide (1%; Ursapharm, Saarbrücken, Germany) and phenylephrine hydrochloride (5%; Ankerpharm, Rudolstadt, Germany), and a lateral canthotomy was created. Hemostasis was achieved with wet-field cautery. After pars plana sclerotomy, a circumscript vitrectomy was performed in the area of the future detachment. Thin glass micropipettes attached to 250 μl glass syringes (Hamilton, Reno, NV) were used to create a retinal detachment by subretinal injection of saline followed by 0.25% sodium hyaluronate (Healon; Pharmacia & Upjohn, Dübendorf, Switzerland) in saline. The retina below the optic nerve head was detached while the retina above the optic nerve head remained attached. After surgery, gentamicin (5 mg) and dexamethasone (0.5 mg) were injected subconjunctivally. The lateral cauthotomy was closed with 5–0 silk sutures, and atropine (1%) eye drops were instilled into the conjunctival sac. After a survival time of 7 days, the animals were anaesthetized as described, the eyes were excised, and the animals were killed by i.v. T61 (embutramid mebezonium iodide; 0.65 ml/kg body weight; Hoechst, Unterschleißheim, Germany).

Isolated retinas were fixed in 4% paraformaldehyde for 2 h. After several washing steps in buffered saline, the tissue was embedded in saline containing 3% agarose (w/v), and 70-μm thick slices were cut by using a vibratome. The slices were incubated in 5% normal goat serum plus 0.3% Triton X-100 in saline for 2 h and, subsequently, in primary antibody overnight at 4 °C. After washing in 1% bovine serum albumin in saline, the secondary antibodies were applied for 4 h at room temperature. The following antibodies were used: mouse anti-vimentin (1:500; V9 clone, Sigma-Aldrich, Taufkirchen, Germany), anti-ET_A (1:100; Alomone Labs, Jerusalem, Israel), anti-ET_B (1:100; Alomone), Cy3-conjugated goat anti-rabbit IgG (1:400; Dianova, Hamburg, Germany), and Cy2-coupled goat anti-mouse IgG (1:400; Dianova).

In control retinas, the immunoreactivity for vimentin was most strongly expressed in the innermost retinal layers (nerve fiber and ganglion cell layers) that reflects the prominant expression of vimentin by retinal astrocytes and endfeet of Müller cells (Fig. 1A and B). In addition, Müller cell fibers that pass through the inner retinal layers displayed faint staining for vimentin. In retinal tissue that was detached for 7 days, the vimentin labeling of Müller cells increased strongly when compared to control, with labeling of Müller cell fibers from the inner to the outer limiting membranes.

In control retinas of the pig, the immunoreactivity for ET_A was expressed in the innermost retinal layers and in the outer plexiform layer (Fig. 1A). At 7 days after detachment, immunoreactivity for ET_A was strongly expressed in the blood vessels of the inner retina. In control tissue, immunoreactivity for ET_B was expressed within the ganglion cell and nerve fiber layers, as well as by Müller cell fibers (Fig. 1B). In respect to Müller cells, co-staining of vimentin and ET_B immunoreactivities was observed at the fibers that pass through the inner plexiform and nuclear layers, and through the outer retina. Additionally, the outer limiting membrane was ET_B immunopositive. In addition to ganglion cell bodies that express immunoreactivity for ET_B, it is likely that both the astrocytes, which are localized preferentially around the vessels in the ganglion cell/nerve fiber layers, and the Müller cell endfeet express immunoreactivity for ET_B. In the detached retinas, no apparent alteration of the ET_B immunostaining was observed.

Endothelin-1 is a major regulator of the retinal blood flow that produces vasoconstriction of the retinal microvessels and subsequent decrease of retinal blood flow, predominantly by activation of ET_A receptors [11]. In the present study, we found different expression of ET_A and ET_B immunoreactivities in the porcine retina: In control retinas, immunoreactivity for ET_A was expressed in the innermost retinal layers (likely by retinal astrocytes) and in the outer plexiform layer, while immunoreactivity for ET_B was expressed by the innermost retinal layers, by ganglion cell somata, and by Müller glial cells. Moreover, we found a different regulation of receptor protein expression upon retinal detachment: whereas the immunoreactivity for ET_A was strongly upregulated in retinal blood vessels, the immunoreactivity for ET_B was not altered in its expression after detachment.

The functional significance of the vascular upregulation of ET_A receptors upon detachment is unknown. It has been shown that intravitreal application of ET-1 produces vasoconstriction and retinal ischemia [19]. There is evidence