Increased Endothelin B Receptor Expression Contributes to Retinal Ganglion Cell Death in a Rodent Model of Glaucoma

Raghu Krishnamoorthy, Alena Minton, Hai-Ying Ma, Nitasha Phatak, Shaoqing He, Dorota Stankowska, Ming Jiang, Brett Mueller. UNT Health Science Center, Cell Biology and Anatomy, Fort Worth, TX 76107, United States.

Purpose: ET_B receptors have been suggested to have neurodegenerative role in glaucoma. The purpose of this study was to determine ET_B receptor activation contributes to retinal ganglion cell death in the Morrison's ocular hypertension model of glaucoma.

Methods: IOP elevation was carried out in one eye of adult male Brown Norway rats using the Morrison's method (by injection of hypertonic saline through episcleral veins), while the contralateral eye served as control. Following intraocular pressure elevation, rats were maintained for 2 to 4 weeks and sacrificed. Retinal sections were obtained from control and IOP elevated rat eyes and analyzed for changes in endothelin receptor expression by immunohistochemistry. Colocalization of endothelin receptor immunostaining was carried out with a retinal ganglion cell marker using an antibody to neuritin, which is selectively expressed in retinal ganglion cells. In a different set of experiments, transgenic wild type and ET_B receptor-deficient rats were used for retrograde labeling of retinal ganglion cells (RGCs) using fluorogold. Following retrograde labeling, IOP was elevated in one eye using the Morrison's method (injection of hypertonic saline through episcleral veins), while the contralateral eye served as control. After IOP was elevated, rats were maintained for 2 to 4 weeks and sacrificed. Fluoro-gold labeled retinas were isolated, flat-mounted, photographed using Zeiss LSM-510 confocal microscope, and labeled RGCs were counted to assess their viability.

Results: IOP elevation produced an increase in ET_B receptor expression in the retinal ganglion cells, inner plexiform layer and inner nuclear layer as determined by immunohistochemical analysis. An increased colocalization of ET_B receptors with neuritin was also found mainly in retinal ganglions cells and inner plexiform layer in rat eyes with elevated IOP. Retinal ganglion cell loss after IOP elevation for 4 weeks was attenuated in the ET_B-deficient rats, compared to wild type rats.

Conclusions: Increased ET_B receptor expression was found in retinal ganglion cells following ocular hypertension in rats. Decreased retinal ganglion cell loss in ET_B receptor-deficient rats suggests a causative role of ET_B receptors in neurodegeneration in glaucoma. Since ET_B receptors mediate neurodegenerative effects, ET_B receptor antagonists could be potential neuroprotective agents in the treatment of glaucoma.