Adrenergic receptor function and role in neuroprotection

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INTRODUCTION

Glaucoma is characterized by optic nerve injury with progressive degeneration of retinal ganglion cells (RGCs) and subsequent loss of vision. Current treatments (reduction of intraocular pressure) does not always alleviate damage, so alternative methods of neuroprotection are desired. Brimonidine, a glaucoma-treatment drug, is suspected to have additional neuroprotective effects through its interaction with the receptor Adra2a, but the mechanisms are not well understood. In this project, we use an established glaucoma-bead mouse model and an Adra2a-mutant mouse line to analyze the role Adra2a plays in the effects of brimonidine-mediated neuroprotection.

DESIGN & METHODS

Identifying Adra2a Expression in Mice

1. Single Cell RNA Sequencing (scRNA-seq)
2. RNA-fluorescent in situ hybridization (FISH, Cryosections)
3. Protein Immunohistochemistry (Cryosections)

Experimental Design: Bead-Glaucoma Model

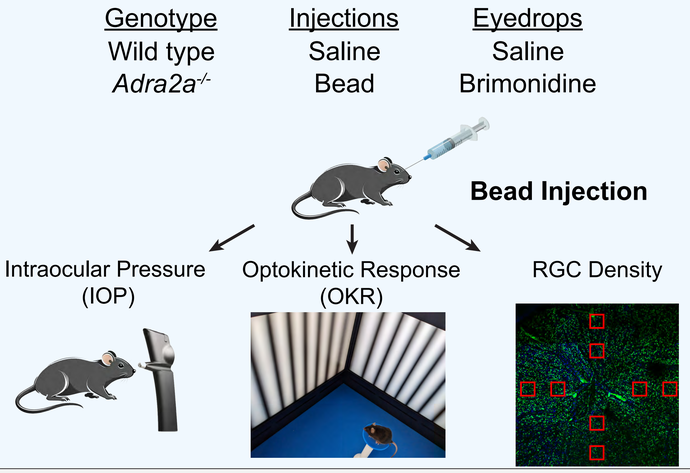

Figure 1. Methods: Both wild type (WT) and *Adra2a*$^{-/-}$ mice were injected with either saline (control) or beads in one eye. Over a period of six weeks, the injected eyes were treated daily with either saline eyedrops (control) or brimonidine. During this period, IOP was measured three times a week. Visual integrity by OKR (spatial frequency and contrast sensitivity) was measured at three and six weeks. After six weeks, the retinas were dissected for whole-mount immunofluorescence using DAPI and an RGC marker (Brn3a). RGC density is measured by counting from two regions (~50% and 75% of retina radii) in each quadrant (red boxes).

RESULTS

Adra2a is Expressed in Horizontal and Ganglion Cells

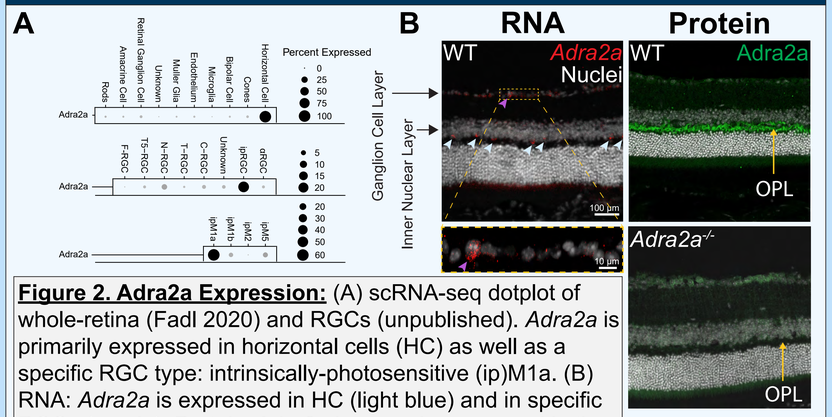

Figure 2. Adra2a Expression: (A) scRNA-seq dotplot of whole-retina (Fadl 2020) and RGCs (unpublished). *Adra2a* is primarily expressed in horizontal cells (HC) as well as a specific RGC type: intrinsically-photosensitive (ip)M1a. (B) RNA: *Adra2a* is expressed in HC (light blue) and in specific RGCs (purple). Protein: Adra2a is strongly expressed in the outer plexiform layer (OPL) but detection in RGCs is uncertain. *Adra2a*$^{-/-}$ mice lack expression in the OPL.

Effect of Brimonidine (Bmd) and Adra2a in Vision Loss

Legend	Saline	Bead	Bead-Bmd
Wild Type	n = 2	n = 2	n = 3
Adra2a$^{-/-}$	n = 2	n = 3	n = 5

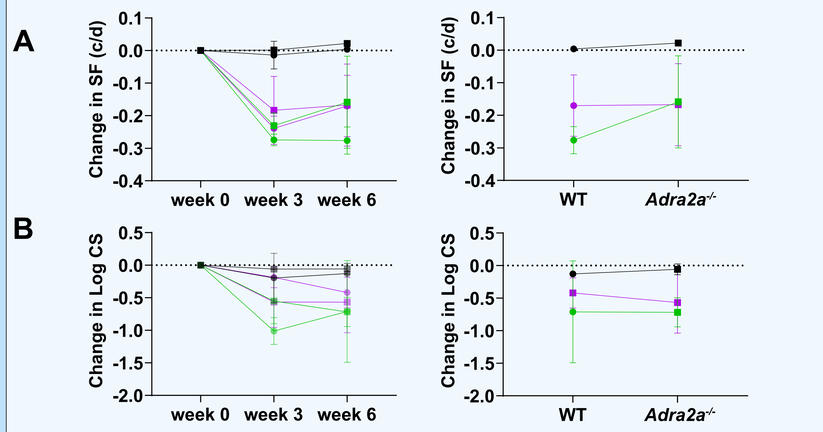

Figure 3. Effect of Brimonidine and Adra2a on Discernment of Spatial Frequency and Contrast: (A) Spatial Frequency (SF) measured in cycles per degree (c/d). (B) Log of Contrast Sensitivity (CS). All measurements are normalized to both the contralateral eye (uninjected) and the 0-week baseline. Average values in brimonidine-treated eyes depict reduced loss of vision but comparisons are not statistically significant.

RESULTS

Effect of Brimonidine and Adra2a on IOP

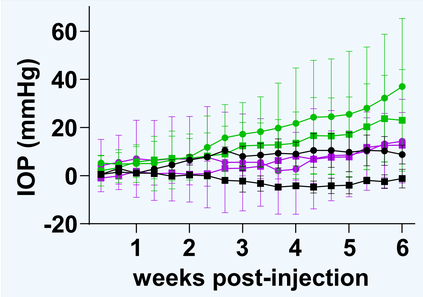

Figure 4. Intraocular Pressure Response to Bead Injection and Brimonidine Treatment: Average IOP readings indicate that bead injection led to IOP increase and this was reduced by brimonidine treatment. No apparent difference is observed between brimonidine treatment in wild type vs *Adra2a*$^{-/-}$ mice.

Effect of Brimonidine and Adra2a on RGC Survival

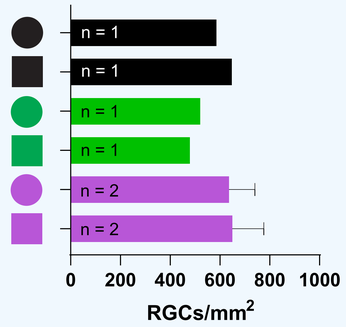

Figure 5. RGC Density: Density is measured by counting the number of RGCs across a total area of ~4.2 mm^2. Preliminary data suggest that RGC density is reduced by bead-injection and is rescued by brimonidine treatment. Differences between wild type and mutant mice are inconclusive.

CONCLUSIONS

Preliminary Conclusions

- In mice, Adra2a is primarily expressed in horizontal cells and ipM1a-RGCs.
- Brimonidine eyedrops may reduce bead-induced loss of vision both in terms of spatial frequency and contrast sensitivity.
- It is unclear whether bead-induced loss of vision or the effects of brimonidine treatment are affected by the presence of functional Adra2a receptor.

Next Steps

- Increase sample size and statistical power.
- Analyze Adra2a gain of function.
- Analyze RGC survival in a type-specific context.

ACKNOWLEDGEMENTS

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