Intrinsic mechanosensation in mammalian retinal ganglion cells is mediated by TRPV4

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INTRODUCTION

Retinal Ganglion cells (RGCs) are immersed within a mechanically active environment in which they must constantly cope with and adapt to hydrostatic pressure and osmotic stress. Elevated intraocular pressure (IOP) can provoke degeneration of retinal ganglion cells (RGCs) in glaucoma, yet the precise mechanisms by which pressure influences the physiology of RGCs remain elusive. The purpose of this project was to identify the molecular mechanism that underlies the mechanosensitive properties of mouse RGCs and to characterize the role of plasma membrane stretch in RGC calcium homeostasis.

METHODS

Animals: C57BL/6J (JAX), DBA/2J (JAX), TRPV4$^{-/-}$ (Dr. Wolfgang Liedtke, Duke U.), and B6.Cg-Tg(*Thy1*-CFP)23Jrs/J mice (Dr. Nicholas Brecha, UCLA).

Immunohistochemical staining (IHC): was performed on 12-14 μm cryosections of PFA-fixed retinas. Images were taken with a Zeiss LSM510 confocal microscope. ICC was performed on 95% methanol/5% acetone-fixed, dissociated retinal cells.

Western Blot: Retinal tissue was homogenized and proteins were denatured. 10 μg of protein was loaded into SDS-gel lanes and blotted to PVDF. After blocking and staining, protein bands were visualized with peroxidase-based chemoluminescence.

Gene expression measurement: RT-PCR was used to determine relative mRNA expression levels of TRPV4 in wild type (WT) and DBA/2J mice.

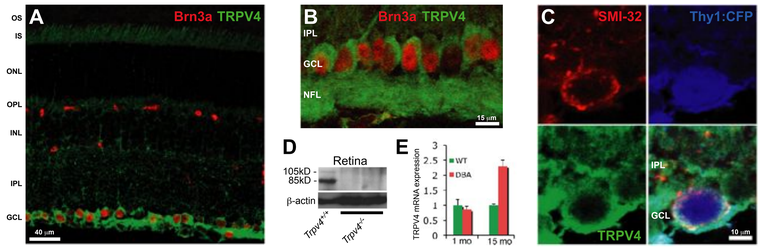
RGC identification: RGCs were identified by position in retinal layers, Thy1:CFP expression, Brn3a-immunoreactivity (ir), SMI-32-ir, and perikaryal diameter > 7μm. RGC vitality was assessed by responsiveness to glutamate application.

Intracellular calcium ([Ca^{2+}]$_i$) measurement: Retinas were isolated and cells dissociated with papain in L-15 Leibovitz medium. Cells were plated, then loaded with 5 μM Fura-2 AM for 30 minutes. The superfusion saline was set to pH 7.4 and osmolarity 280 mOsm. The osmolarity was adjusted by the mannitol concentration. The imaging systems were: Nikon Ti/CoolSnap HQ2 and Nikon E600FN/Cascade 650. Excitation light was from a Lambda DG-4 wavelength switcher. [Ca^{2+}]$_i$ was calculated by calibration for each individual cell, using a Kd value of 224 nM.

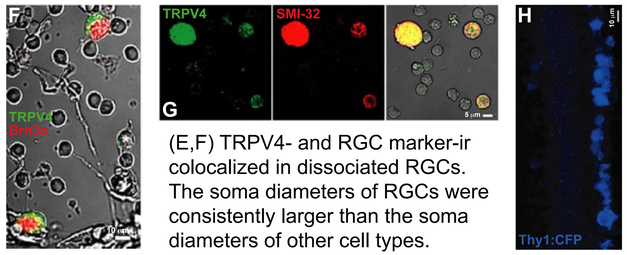
Pharmacological agents: Agonists: glutamate 100 μM, GSK1016790A (GSK) 25 nM, 4α-PDD 30 μM. Non-specific TRP channel blockers: ruthenium red (RR) 10 μM, gadolinium 100 μM (Gd^{3+}). TRPV1 blocker: Capsazepine 5 μM.

IOP manipulation and measurement: IOP was measured in mice anesthetized with Avertine using a tonometer. IOP was measured twice one week prior to injection. Microbeads (or PBS as a control) were injected into the anterior chamber to increase IOP. IOP was monitored with bi-weekly measurements prior to harvesting the eyes.

RGCs express TRPV4

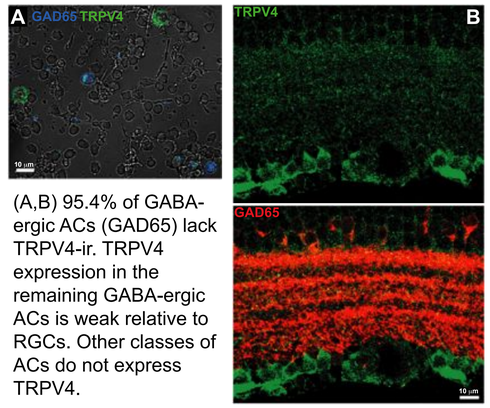


(A,B,C) IHC in retinal slices reveals TRPV4 in the membranes, axons, and proximal dendrites of RGCs. RGCs were identified by Brn3a-ir (nuclei), SMI-32-ir (neurofilament H), and Thy1:CFP expression (A-C,F-H). The TRPV4 antibody recognized TRPV4 protein in wild type but not TRPV4$^{-/-}$ retinas (D). RT-PCR revealed greater TRPV4 mRNA expression in aged DBA mice (E).



(E,F) TRPV4- and RGC marker-ir colocalized in dissociated RGCs. The soma diameters of RGCs were consistently larger than the soma diameters of other cell types.

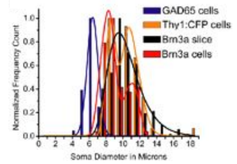
ACs do not express TRPV4



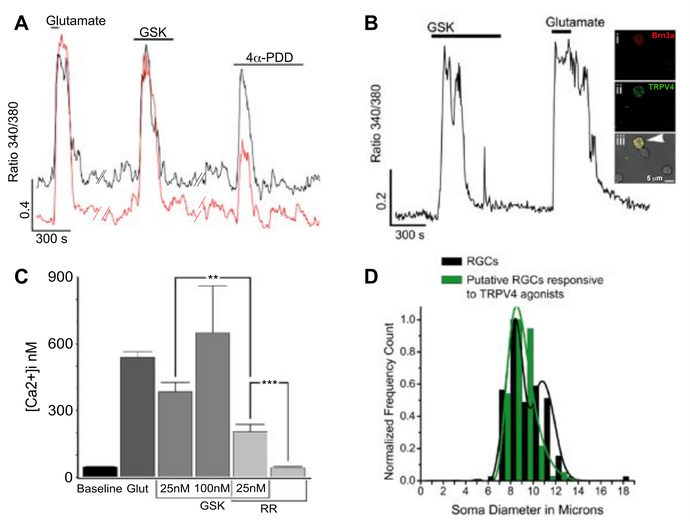
(A,B) 95.4% of GABA-ergic ACs (GAD65) lack TRPV4-ir. TRPV4 expression in the remaining GABA-ergic ACs is weak relative to RGCs. Other classes of ACs do not express TRPV4.

RGCs are larger than ACs

The soma diameter distributions of labeled RGCs does not overlap with the distribution of AC diameters. Soma size can thus serve as a marker for dissociated RGCs.

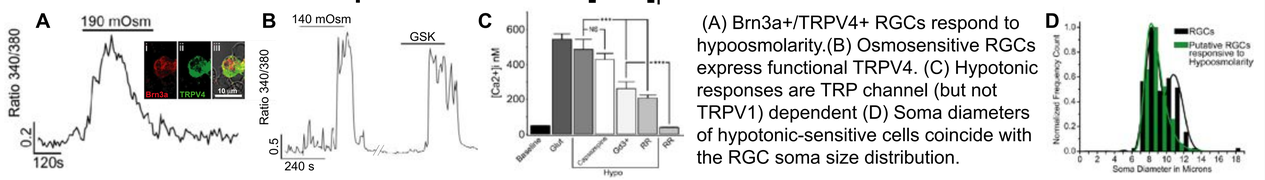


TRPV4 is functional in RGCs



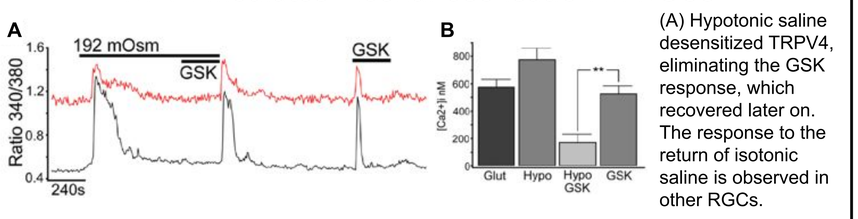
RGCs responded to selective TRPV4 agonists GSK and 4α-PDD with robust increases in [Ca^{2+}]$_i$ (A,B,C). These increases were inhibited by ruthenium red (RR), a TRPV4 antagonist ($p < 0.01$; C). Brn3a+/TRPV4+ RGCs respond to GSK (B).The soma diameter distribution of TRPV4-responsive cells is nearly identical to that of Brn3a+/Thy1:CFP+ RGCs (D).

Osmotic pressure evokes [Ca^{2+}]$_i$ increases in RGCs



(A) Brn3a+/TRPV4+ RGCs respond to hypoosmolarity.(B) Osmosensitive RGCs express functional TRPV4. (C) Hypotonic responses are TRP channel (but not TRPV1) dependent (D) Soma diameters of hypotonic-sensitive cells coincide with the RGC soma size distribution.

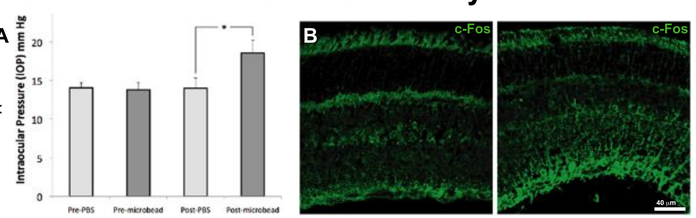
TRPV4 mediates mechanosensation in RGCs



(A) Hypotonic saline desensitized TRPV4, eliminating the GSK response, which recovered later on. The response to the return of isotonic saline is observed in other RGCs.

Elevated IOP increases RGC activity

(A) Injection of microbeads into the anterior chamber increased IOP relative to the PBS control ($p < 0.05$). (B) Neuronal activity, was assessed with the level of cFos. expression.



CONCLUSIONS

These results demonstrate that mouse RGCs are intrinsically mechano-sensitive. Molecular transduction of mechanical stimuli involves TRPV4, a polymodal pressure- and osmo-sensitive channel which provides prominent modulation of RGC calcium homeostasis and excitability. Our findings may have implications for blinding diseases associated with pathological changes in intraocular pressure.

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