Agents modulating Acid-Sensing Ion Channels (ASICs) in human primary brain and retinal astrocytes
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INTRODUCTION
Glaucoma is the second leading cause of blindness worldwide. Acid-sensing ion channels (ASICs) increased in animal models of glaucoma but the exact mechanism is still unknown. Several cytokines and hormones such as steroids (DEX), endothelin-1 (ET-1), tumor-necrosis factor-alpha (TNFα) and transforming growth factor-beta-2 (TGFβ2) are elevated in glaucoma or even induce glaucoma. The purpose of these experiments is to evaluate the effects of DEX, ET-1, TNFs, and TGFβ2 on ASICs' gene expression. Both human primary brain and retinal astrocytes were treated for 24hr with DEX (100 nM), ET-1 (250 nM), TNFα (10ng/ml), or TGFβ2 (10ng/ml) followed by RNA isolation, cDNA synthesis and real-time PCR to quantify changes in ASIC1, ASIC2, and ASIC3 gene expression. Only TNFα synthesis and real-time PCR to quantify changes in ASIC1, ASIC2, and ASIC3 gene expression. Both decreased both TGFβ2 and TNFα ASICs 2 and 3. Bar = 20 µM

METHOD
Cells: The human primary retinal astrocytes were treated with the Cell-lines: Human primary retinal and brain astrocytes were purchased from Sciencell Inc. (Carlsbad, CA). Cells passages 3-7 were used in the current study. Cells were grown in DMEM-media containing 10% FBS and antibiotics.

Study design: Cells were serum starved overnight. On the following day, the following day cells were treated with; deoxycorticosterone (plasma, 100 nM), endothelin-1 (ET-1, 250 nM), TNFα (10ng/ml), or TGFβ2 (10ng/ml) for 24hr by follow RNA isolation or 48hr followed by immunocytochemistry analysis.

Real-Time analyses of ASIC1, ASIC2, and ASIC3 cytospin pH levels (pHOR): Cells were washed with 1X PBS. Total RNA was isolated from cells with TRIzol (Life Technol) and visualized using a confocal microscope (Zeiss LSM 510, Carl Zeiss, Thornwood, NY) with a ×40 objective lens.

RESULTS
Immunocytochemistry on human retinal astrocytes: Cells plated on coverslips were washed 3x in ice-cold PBS followed by fixation for 3 min at 4°C with ice-cold methanol-acetone (1:1). Cells were then washed 3x in 1X PB then incubated with 5% normal goat serum/5% BSA in PBS for 1hr at 4°C. Following blocking, coverslips were incubated in mouse anti-ASIC1 (1:50, Antibody Research Inc), goat anti-ASIC2 (1:50, sc-22333, Santa Cruz Inc, CA), and rabbit anti-ASIC3 (1:50, sc-63866, Santa Cruz Inc, CA) for 4hr, at room temperature to assess levels of ASIC1, 2, and 3 in retinal astrocytes. Coverslips were washed 3 times in PBS (5 min each) and incubated in Alexa Fluor 488 donkey anti-mouse IgG (cat# A21202, Life Technologies) and Alexa Fluor 647 donkey anti goat IgG (cat# A21447, Life Technologies) for 1hr at RT. All secondary antibodies were used at 1:1000 dilution. After three washes, sections were mounted with mounting medium (Life Technologies) and visualized using a confocal microscope (Zeiss LSM 510, Carl Zeiss, Thornwood, NY) with a ×40 objective lens.

Fig 1. Effect of DEX and ET-1 on ASICs' protein levels. While ET-1 decreased both ASICs 2 and 3, DEX appears to only reduce ASIC2. To check for nonspecific binding, cells were treated without primary antibodies followed by adding secondary antibodies (NP). Bar = 20 µM

Fig 2. Effect of TGFβ2 and TNFs on ASICs' protein levels. Both decreased both TGFβ2 and TNFα ASICs 2 and 3. Bar = 5 µM

Fig 3. Effects of known agents on ASIC1 (3A) and ASIC2 (3B) gene expression.

CONCLUSIONS
ASICs' gene expression is differentially modified in human primary brain and retinal astrocytes. TNFα-induced reduction of ASICs 1 and 3 may suggest impaired pH regulation in astrocytes. Future studies are needed to further confirm current findings.

REFERENCES

ACKNOWLEDGEMENTS
This work was completed thanks to the SMART summer program, the North Texas Eye Research Institute, and Dr. Adnan Dibas.
Funded a grant from Brightfocus Foundation to Dr. Adnan Dibas and by the Department of Health and Human Service, National Institute of Health, National Heart, Lung and Blood Institute, SMART Grant SR25HL007786-25 to Dr. Jamboor K. Vishwanath.