The role of α2δ1 in mediating extracellular signaling in retinal cells

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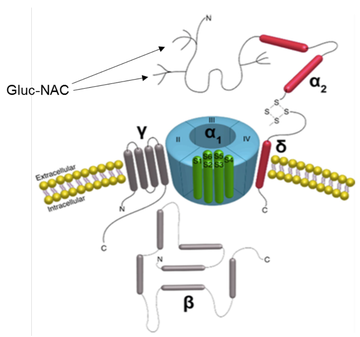
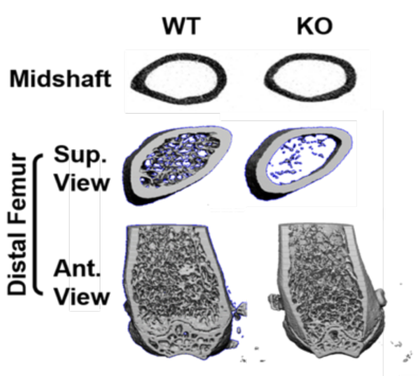
ABSTRACT

Voltage sensitive calcium channels (VSCCs) are key components in coordinating extracellular signaling that influence calcium (Ca2+) influx and subsequent downstream signaling in a variety of tissues including bone. Previous work involving inhibition or genetic deletion of VSCCs impaired bone formation and responses to loading. These studies focused on the pore-forming α1 subunit of VSCCs. In addition to α1, VSCC complexes contain auxiliary subunits, including α2δ1, that influence channel function. The role of the α2δ1 subunit of VSCCs are underappreciated in specific types of neuronal tissue, particularly retinal cells. Clinically, the α2δ1 subunit is pharmacologically targeted by gabapentin, blocking the channel to increase synaptic GABA concentration to aid in modulating nociception. Recently, gabapentin treatment has been associated with cases of macular edema. The purpose of our study was to characterize in vitro models of retinal α2δ1 expression and examine if changes in intraocular pressure are mediated via VSCCs. Two immortalized rat retinal cell lines, R28 and RMC-1, were characterized for α2δ1/4 expression under both static and oscillatory fluid shear stress (OFSS) conditions. Initial Western blot data support that both R28 and RMC-1s are responsive to OFSS as quantified by the phosphorylation of ERK. Ongoing studies are focused on further characterization of the R28 and RMC-1 cells lines for changes in α2δ1/4 expression under static and OFSS conditions. Future studies will include treating R28 and RMC-1 cells with gabapentin to examine its capability of modulating α2δ1 function in retinal cells.

BACKGROUND



Cartoon of the general structure of a voltage-sensitive calcium channel (VSCC) with the exofacial domain of $\alpha_2\delta_1$ circled in green and N-acetyl glucosamine residues (Gluc-NAC) emphasized with black arrows. Thompson et al., 2011.



Femoral uCT Analysis: Observational changes in trabecular and cortical bone were seen in femoral uCT images of distal end and midshaft of 22 week-old KO mice compared to age and sex-matched controls. A significant reduction in femoral bone volume divided by total volume was observed in male but not female KO mice compared to age and sex-matched controls.

HYPOTHESIS

α2δ1 PARTICIPATES IN MODULATING Ca2+ SIGNALING IN RESPONSE TO GABAPENTIN TREATMENT

STUDY GOAL:

Characterize different retinal cell lines to investigate α2δ1 role in modulating Ca++ signaling

RESULTS

Characterization of RMC-1

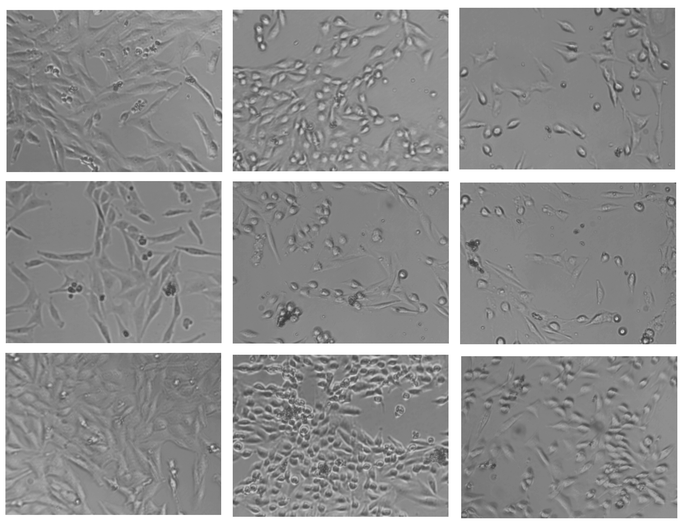


Figure 1a: The rmc-1 cells were cultured on three 6-welled, flat-bottom, non-coated plates with each left static, shaken at 200rpm for one hour, or shaken at 250rpm for two hours. A photo of each was captured after using light microscopy at 10X magnification.

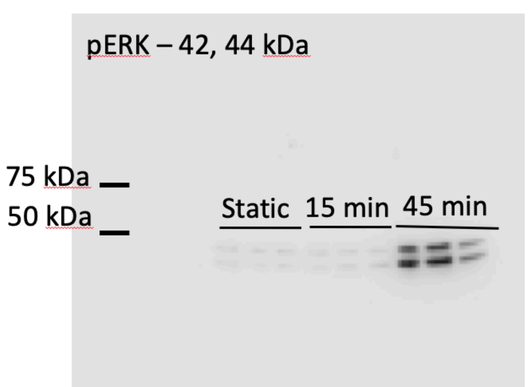


Figure 1b: The rmc-1 cells were similarly cultured and harvested for western blot analysis after static, 15 minute, and 45 minute time points. The gel was probed for pERK, with the longest fluid-stress time point showing most pERK induction.

RESULTS

Characterization of R28

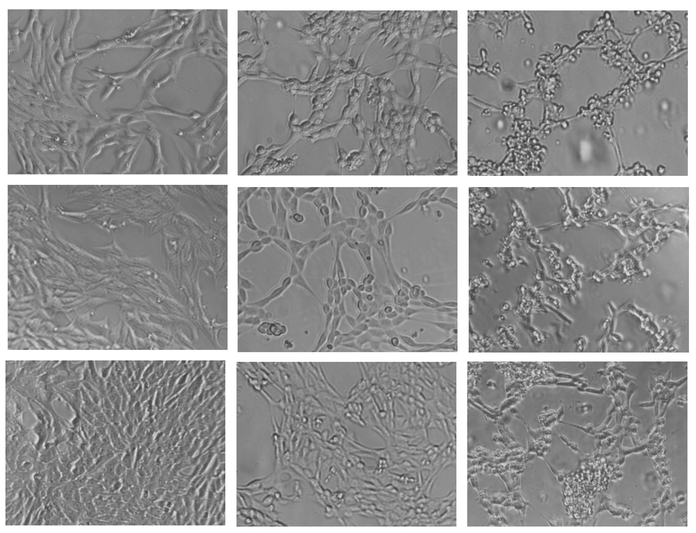


Figure 2a: The R28 cells were cultured on three 6-welled, flat-bottom, non-coated plates with each left static, shaken at 200rpm for one hour, or shaken at 360rpm for two hours. A photo of each was captured after using light microscopy at 10X magnification.

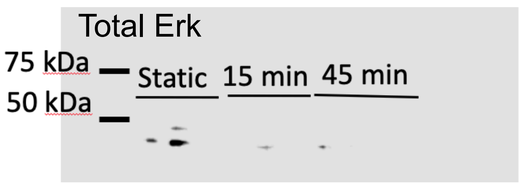


Figure 2b: The R28 cells were similarly cultured and harvested for western blot analysis after static, 15 minute, and 45 minute time points.

FUTURE DIRECTIONS

- Repeat qPCR analysis of α2δ1, α2δ2, α2δ3, α2δ4
- Repeat OFSS experiments and Western blots: pErk, Total Erk, B-actin
- Examine Ca++ release in RMC-1 and R28 cells in response to OFSS
- Treat retinal cells with Gabapentin and measure intracellular Ca++ changes

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