Identifying KIF Subtype that Mediates Axonal Targeting of Kv7 Channels

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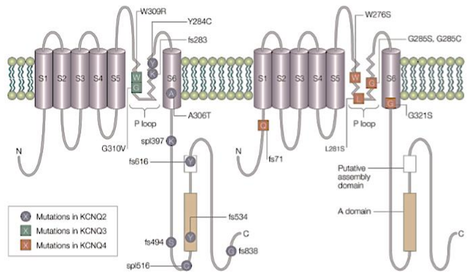
PRECS Phenotypic Plasticity Research Experience for Community College Students

Introduction

- Epilepsy, a brain disorder that effects about 2% of the population, is characterized by recurrent, unprovoked seizures.
- A seizure is a transient disruption of brain function due to abnormal and excessive electrical charges.
- Early-onset Benign Familial Neonatal Epilepsy (BFNE) and Epileptic Encephalopathy (EE), are associated with mutations in neuronal KCNQ/Kv7 channel subunits Kv7.2 and Kv7.3.



- Kv7 channels are voltage-dependent potassium channels. Enriched at the axonal plasma membrane, they pump potassium ions out of the neurons and inhibit repetitive or burst firing of action potentials.
- A single neuronal Kv7 channel is a heterotetramer composed of two Kv7.2 and two Kv7.3 subunits.
- BFNE and EE mutations in Kv7.2 and Kv7.3 lead to decreased surface expression along the axon, which means less potassium ions are moved across the axonal membrane where action potentials are generated and propagated. This prevents the neuron from returning to its resting potential and allows repetitive action potentials indicative of a seizure.

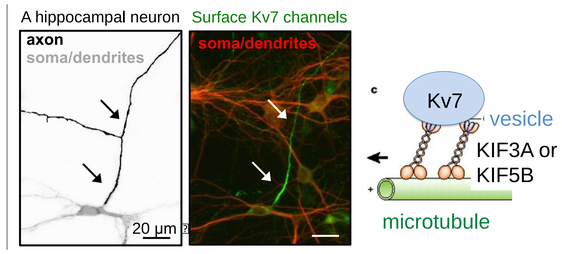
Project Description

The purpose of this project is to uncover the molecular mechanism by which Kv7 channels are targeted to the axonal surface and enriched at the axonal initial segment (AIS).

Since epilepsy mutations in Kv7 channels reduce this axonal targeting, understanding the mechanism underlying axonal targeting could provide therapeutic targets to treat epilepsy. Two motor proteins KIF3A and KIF5B are shown to target other potassium channels such as Kv1 to the axon. Here, we are investigating to test if KIF3A and KIF5B mediates targeting of Kv7 channels to the axons.

Hypothesis

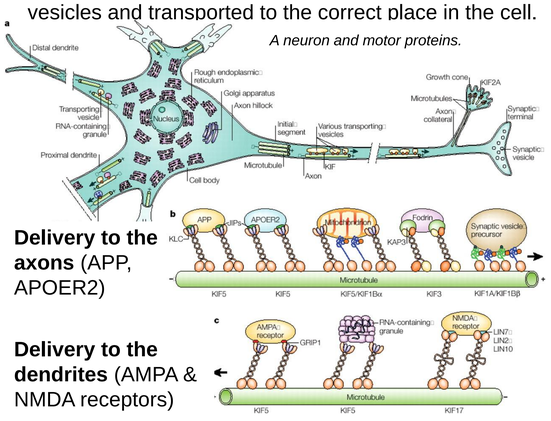
Overexpression of KIF3A and KIF5B proteins will lead to increased surface expression of neuronal Kv7 channels in the axon of hippocampal neurons.



Enrichment of Kv7 channels on the axonal surface (Arrows mark the main axon).

Background

- Transmembrane proteins (such as Kv7 subunit) are synthesized at the rough ribosomes in the ER located at the soma. Upon processing through Golgi and TGN (glycosylation and cleavage), they are loaded into the vesicles and transported to the correct place in the cell.



Delivery to the axons (APP, APOER2)

Delivery to the dendrites (AMPA & NMDA receptors)

- Kinesin superfamily proteins (KIFs) are motor proteins that move along the microtubules of a cell, carrying vesicles, which are small transport cargos for proteins
- Different proteins are transported to axons and dendrites via specific KIF proteins.
- KIF3A targets Kv1 ion channels to the axon and KIF5B targets Kv3 ion channels to the axon.

The Research Methods

- **Mammalian expression constructs:**

Each type of KIF protein used is tagged with YFP (yellow fluorescent protein) to visualize KIF in the transfected neurons. The HA tag (which is a chain of 9 amino acids) was introduced to the extracellular domain of Kv7.3 to label and visualize surface population of Kv7.3/Kv7.2 channels.

- **Hippocampal neuronal culture and transfection:**

Hippocampal neuronal culture was prepared from embryonic 19 day old rat hippocampi. At 5 DIV (days *in vitro)*, rat hippocampal cultured neurons were transfected with either YFP-KIF3A or YFP-KIF5B, as well as HA tagged Kv7.3 and Kv7.2. The neurons were allowed to incubate at 37ºC incubator for 48 hours.

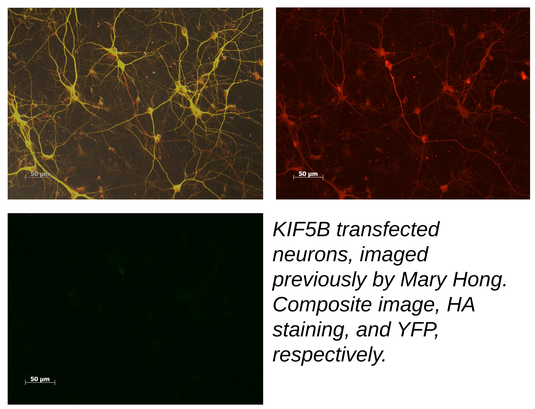
- **Surface Immunostaining:**

The neurons were fixed and washed with PBS. Neurons were blocked with 10% normal donkey serum for 1 hr, and then incubated without permeabilization with anti-HA antibody to label surface HA-Kv7.3/Kv7.2 channels. After washing with PBS, surface channels were visualized with Alexa598-conjugated secondary antibodies.

- **Permeabilized Immunostaining:**

After completing surface immunostaining, the neurons were briefly fixed again, washed with PBS, and incubated with 0.1% TritonX-100 to permeabilize the neurons. Neurons were incubated with anti-Kv7.2 antibody and anti-GFP antibody. After washing with PBS, Kv7.2 and GFP proteins were visualized with Alexa680 and Alexa488-conjugated secondary antibodies, respectively. The coverslips were mounted on slides.

- **Epifluorescent microscopy**



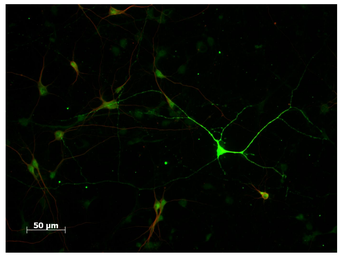
KIF5B transfected neurons, imaged previously by Mary Hong. Composite image, HA staining, and YFP, respectively.

- **Trouble shooting**

We had very low expression of YFP-tagged KIF proteins. Because of this, most of our work was troubleshooting and verifying our YFP-tagged KIF constructs using DNA restriction digest and western blotting.

Results

A neuron was successfully transfected with YFP-KIF5B (green).



Future Work

Continuing from here, more experiments need to be carried out to obtain successful transfections with KIF along with HA-Kv7.3 and Kv7.2. This will allow us to see the localization of the KIF proteins and Kv7 channels.

It will also be necessary to knock down the endogenous KIF proteins to see if the Kv7 channels are targeted less to the axonal surface. This will be done using siRNA constructs, which will silence the genes that code for the KIF proteins.

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