CHM 197
Friday, July 15th 2016
10:00 AM
PhD Final Defense

Development of cellular high-throughput assays to determine the electrophysiological profile of $GABA_A$ receptor modulators for neurology and immunology

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Gamma (γ) -aminobutyric acid (GABA) is the major inhibitory neurotransmitter found in the mammalian central nervous system. Its effect stems from its ability to cause the opening of ion channels which causes an influx of negatively charged chloride ions or an efflux of positively charged potassium ions. This hyperpolarization of the neuron lowers the threshold for neuronal firing. This has an overall inhibitory effect on neurotransmission, decreasing the excitability of the neuron and diminishing the likelihood of a successful action potential occurring. The ligand-gated $GABA_A$ receptor is a pentameric receptor containing two binding sites for GABA and once bound, the channel opens to allow the influx of chloride ions. However, $GABA_AR$ not only contains GABA binding sites but also binding sites that modulate the actions of GABA. This includes the benzodiazepine (BZD)-binding site which occurs at the α and γ2 interface.

Over the past decade, there has been an emerging understanding of the specific subunit composition which mediates the diverse spectrum of BZD pharmacological effect which has generated great interest in developing α-subtype selective drugs. There are at least nineteen different individual $GABA_AR$ subunits that assemble the 5-subunit structure into different combinations to form the native receptor (α1-6, β1-3, γ1-3, δ, and minor subunits).

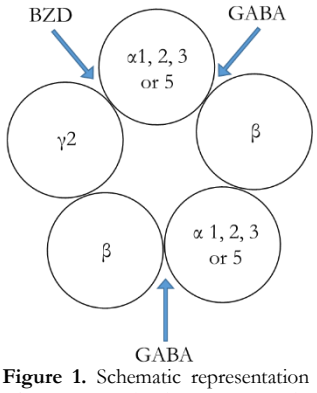

Figure 1. Schematic representation of $GABA_AR$ subunit arrangement in the synapse.

Electrophysiological techniques are critical in determining the enhancement of chloride conductance and calculating potency and efficacy of the drugs but data collection is limited by slow throughput. Herein the development of higher throughput cellular assays to determine BZD subtype selectivity is described. First, an assay was created and optimized using transiently transfected cells on automated patch clamp. However, this assay suffered from variable reproducibility. Next, receptor subtypes were recombinantly expressed in stable cell lines using a single plasmid and antibiotic. These cells can be reliably used to determine subtype specificity of compounds. The overall potency and efficacy of the drugs were also tested on commercially available human neuronal induced pluripotent stem cells (IPSC) which would more accurately reflect the mixture of receptor subtypes natively expressed on human neurons. Next a fluorescence assay, which utilizes an enhanced yellow fluorescent protein that quenches in the presence of selective anions, was optimized and tested in order to determine if the assay was suitable to perform structure activity relationship studies. Finally, the $GABA_AR$ was found to be present in leukocytes and multiple cell sources were tested to determine their subunit composition and electrophysiological behavior.

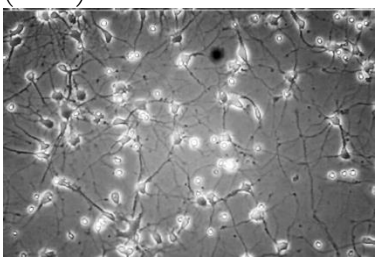

Figure 2. Human neuronal induced pluripotent stem cells

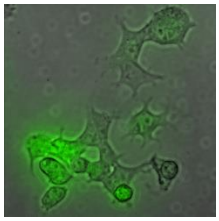

Figure 3. Fluorescence imaging of eYFP