Glutamate Receptor Crosstalk in Alzheimer’s Disease

Introduction:

Alzheimer’s disease (AD) is ranked as the sixth leading cause of death among adults according to the Alzheimer’s Association Fact Sheet of 2021. It is also predicted that with the growing population and extended life expectancy, the number of individuals affected by dementia is expected to grow threefold by 2050. The limitation of current drug treatments available to combat the disease highlights our need to advance our understanding of the AD pathophysiology. With this understanding, we will better be able to identify and develop new therapies and drug treatments against AD.

Background:

The N-methyl D-aspartate (NMDA) receptor channels regulate functions of the brain by activating a series of calcium-mediated signaling pathways. Dysfunction of this pathway has been associated with AD, where the increase in activity of NMDA receptors is associated with the neurotoxicity and neurodegeneration that is observed in AD. Because of this observation, some treatments of AD include NMDA receptor antagonist response to decrease the receptor’s activity and therefore decrease the possibility of neurotoxicity and neurodegeneration that can result from this pathway. Amyloid Beta protein (Aβ) is a naturally occurring protein in the brain and is highly associated with AD. The accumulation of Aβ oligomers leads to the formation of plaque between neurons, disrupting cell function and influencing AD. mGluR5, an essential protein of Aβ...
oligomers, has been suspected of changing NMDA receptor signaling in response to the drug treatment though the mechanism of this process is not understood.

**Experimental Goals:**

We will investigate the NMDAR response with the addition of Aβ oligomers. The project will image Ca\(^{2+}\) influx using the Fluo-8 Calcium Flux Assay Kit in SH-SY5Y cell. This kit, from Enzo Life Sciences, is a fluorescence-based assay for detecting intracellular calcium mobilization in cells. After growing the cells and treating them, the calcium influx will be measured by reading the fluorescence intensity using a microplate reader at multiple intervals. Our hypothesis for this experiment will show that the binding of Aβ to mGluR5 will enhance NMDA receptor activity.

**Methods:**

*Cell Culturing:*

In this experiment, SH-SY5Y cells were used. These types of cells can be used for many types of neurological studies including those related to neurodegenerative processes, neurotoxicity, and neuroprotection. The cells were then thawed, placed in a T-25 along with growth media and incubated at 37 °C along with 5% CO\(_2\) to help the pH of the cell culture and allow the cells to grow. Cells are monitored for confluences and growth media is replaced as needed.

When ready to use the cells for the experiment, the growth media is removed from the T-25 flask that has been incubating. Once the growth media is moved, 1 mL of trypsin is added to detach the cells from the surface of the T-25 flask. The flask is allowed to sit with the trypsin within with occasional light tapping for 6 minutes to ensure that all cells within the T-25 flask lift successfully. After the 6 minutes, 3 mL of growth media is added to neutralize the trypsin. Then a total of 1 mL of the cell suspension solution is added to 4 different centrifuge tubes. The tubes are then centrifuged at 1400 rpm for 5 minutes. The centrifuge tubes were then removed, and the media removed from each tube leaving behind the cells that gathered to the bottom of each tube. The cells within each tube were then resuspended by adding 800 μm of fresh media and were then aspirated within the tubes. This allows the clumps of cells to be broken up. The solution from each of the four tubes are then transferred into a conical tube and further aspirated. 10 μm of the cell solution was then obtained and loaded into a hemocytometer for counting. The number of cells
within the solution is then counted and transferred to a 96 well plate. 1000000 cells are then plated into each well used on the 96 well plate.

After the cells are plated on the 96 well plate, the plate is then incubated at 37 °C and 5% CO₂ for 24 hours to allow the cells to attach to the bottom of each well within the plate. After the 24-hour incubation, the growth media is then removed, and 100 m of FBS-free media is added to each well that is in use within the plate. This will allow the cells to be starved and ready to be treated the following day for assay. The cells within the 96 well plate are then incubated again at 37 °C and 5% CO₂ for 24 hours. After this incubation period, the cells will be ready to be treated with AB peptides and Ca²⁺ assay.

**AB preparation**

Aβ stock is obtained from the freezer and diluted to a 5 mM concentration by adding DMSO. FBS-free culture media is then added to the AB preparation to reach 100 uM concentration. The preparation is then vortexed from 1 minute to dissolve within the solution. The solution is centrifuged for a few seconds. The prepared Aβ is then placed in the fridge for 24 hours to oligomerize. After this period this solution can be used for drug treatment.

**Ca²⁺ Influx Assay**

Reagent A preparation can then be done. 100 μL DMSO is added to a vial of 300 nmol of reagent A, Fluoforte dye, to completely dissolve it. An assay buffer is then prepared by mixing 4.5 mL of reagent C, HHBS, with 0.5 mL of reagent B, dye efflux inhibitor. A dye-loading solution that contains the Ca²⁺ solution can then be prepared. To make this preparation, mix 5 μL of reagent A with 5 mL of the assay buffer. The mixer can then be kept table for 2 hours at room temperature. 100 μL of dye-loading solution is then added to each well that is in use. The 96-well plate is then incubated at 37 °C and 5% CO₂ for 1 hour. After the incubation period, the cells are then treated as follows.

The plate was then placed in the synergy H1 microplate reader and results were obtained.

We have our control wells that contain only cells, and wells with only cells and Aβ and we are able to observe how Aβ normally interact with cells. MK is an NMDA receptor antagonist. With the wells that contain only cells and MK, we are able to see how an NMDA receptor antagonist affects normal cells. MTEP is an mGlu5 antagonist. CHPG is a mGlu5 agonist. With the cells that contain cells, MK and CHPG, to see if the CHPG can activate mGlur5 to modulate
Results and discussion:

Figure 2: Levels of calcium influx between different treatment combinations

Data represent the average and standard error of two independent experiments (each triplicate). P < 0.05 drug treatment vs. ANOVA analysis with Tukey’s post-test

Figure 3: Levels of calcium influx is read, every 10 minutes over the course of 60 minutes

Data represents the average and standard error of two independent experiments (each triplicate). P < 0.05 drug treatment vs. ANOVA analysis with Tukey’s post-test
Based on figures 2, this experiment did not produce a significant fluctuation of Calcium influx. There was a slight increase of Ca\(^{2+}\) influx for the cells treated with Aβ, from 0 to 20 minutes (Figure 3). This is followed by a decrease in Ca\(^{2+}\) influx from 30 minutes to 40 minutes. A slight increase in Ca\(^{2+}\) influx was observed between 40 minutes and 50 minutes, followed by a decrease once again after 60 minutes. Data showed minimal change in Ca\(^{2+}\) influx across treatment combinations, which was not expected. Cells that were treated with MK, an NMDA antagonist, had a very slight increase in Ca\(^{2+}\) influx which is not the expected results from adding this drug to the cells. The results obtained data does not support the proposed hypothesis. However, I believe that going forward this procedure would benefit from allowing Aβ and the other drug treatments to incubate with the cells for 24 hours before having the fluorescent dye added to assess the Ca\(^{2+}\) influx. Allowing the cells to sit for a longer period of time with Aβ and the drug treatments may allow for the pathology of Aβ and the drug treatments to better take place within the cells and produce a greater variety of results that can be observed.

**My Experience:**

As a student already interest in medical school, the opportunity this research provides to take a glimpse into the research that goes into my dream future career is fascinating. The OUR grant allowed me to gain laboratory experience that I otherwise would not have been able to, due to my major being chemical engineering. I received training and guidance in learning the procedure for my experiment. I was also challenged with interpreting the results from the experiment and gained a more in-depth understanding of the research project significance. This process has sparked my interest more into the research side of medicine. While I still want to be a clinician, I hope that in the future I have the opportunity to also incorporate research into my professional career. While the idea of presenting research in front of a large group of people is incredible nerve racking, my enjoyment of being in the lab and gaining these new experiences makes it worthwhile. I am thankful for the opportunity and hope that I can continue my research journey here at Lamar University. I am thankful that Lamar offers ample opportunities for undergraduates to get involved in research, especially since the idea of conducting and presenting research seemed improbable to me as a freshman. It is inspiring to look back and see how much I have grown over the years.
Bibliography:


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