Penn Undergraduate Research Mentorship

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Glycogen Synthase Kinase-3 and the Mechanism of Lithium Sensitivity

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Abstract:

Lithium has been used to treat bipolar disorder and unipolar depression for many years and has proven to be very successful in preventing suicide (Snitow et. al, 2021). Low dose lithium has also been proposed to slow the development of dementia. Lithium inhibits the protein kinase Glycogen Synthase Kinase-3 but also inhibits metabolic enzymes such as inositol monophosphatase, phosphoglucomutase, and fructose 1,6 diphosphatase. It therefore remains unclear which target of lithium mediates the therapeutic response. The goal of this project is to use molecular modeling coupled with wet bench experiments to identify a GSK-3 mutant that is resistant to lithium and then use this mutant to test whether the biological and behavioral effects of lithium are mediated through GSK-3 inhibition.

Objectives/Hypothesis:

The objective of the project is to find a GSK-3 (Glycogen Synthase Kinase-3) mutant that is resistant to lithium. There are two main hypotheses for why GSK-3 is sensitive to lithium. The first is that Lithium competitively inhibits GSK-3 by taking the same place as magnesium in the GSK-3 enzyme. By taking magnesium's place, lithium can effectively stop the enzyme from functioning properly. The second hypothesis is that lithium interacts with GSK-3 independently of the magnesium site and changes the enzyme activity by altering its shape or behavior.

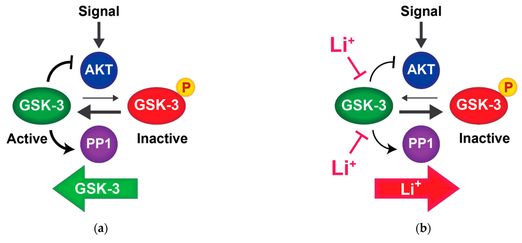

Figure 1: A diagram showing the activity of GSK-3 when not in the presence of lithium and when in the presence of lithium, borrowed from Snitow et. al, 2021. The signal used in both of these cases is constant. In the picture on the left, GSK-3 inhibits AKT and activates PP1, which increases its own activity; in the picture on the right, lithium inhibits GSK-3 and disrupts the AKT and PP1 feedback circuits (Snitow et. al, 2021).

Results:

So far, we have yet to find a mutant GSK-3 that is resistant to lithium. Below, I've included the results from testing the DNA concentrations of plasmids encoding GSK-3 with point mutations (Figure 2). Although several factors could've skewed the results of the experiments such as pipetting accuracy, incorrect centrifuge timing, etc., it seems that the concentrations for the GSK-3 mutants is variable. This information will be useful for the next phase of the project, which involves treating certain GSK-3 mutants with lithium. We also expressed wild-type GSK-3β in human embryonic kidney (HEK293T) cells and then performed western blots to detect GSK-3β as well as two known substrates for GSK-3, glycogen synthase (shown as the phosphorylated form) and β-catenin (Figure 3). β-actin is a loading control. The data shows that GSK-3 protein expression increases with increasing amounts of plasmid and that phosphorylation of glycogen synthase also increases, as expected.

DNA Concentrations for GSK-3 Mutants

Mutation	Mini-Prep Concentration (µg/µL)	Midi-Prep Concentration (µg/µL)
D264T	0.17	0.54
C218G	0.15	0.1
G254A	0.07	0.06
E211A	0.40	0.72
C207A	0.19	0.56
E97A	0.24	0.85
K205A	0.23	0.85
C178A	0.13	0.56
F229A	0.29	0.16
V139A	0.11	lost pellet
L153A	0.33	0.78
L130A	0.69	0.06
L132A	0.22	1.49
F293Q	0.57	0.1
hGSK-3B	0.05	0.16

Figure 2: Chart containing Glycogen Synthase Kinase-3 mutant concentrations using both mini-prep and midi-prep protocols. Glycerol stocks were made with all the listed mutants.

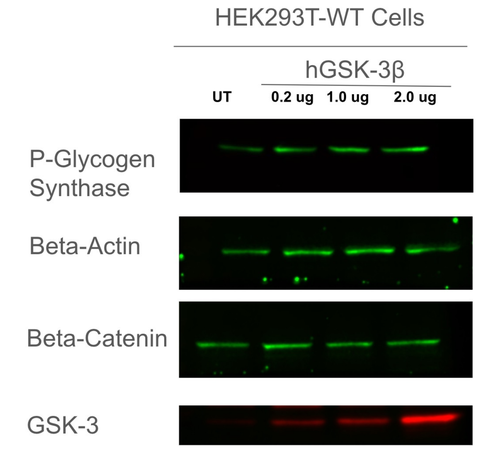

Figure 3: Western blot analysis of HEK293T-WT cells transfected with 0.2 µg, 1.0 µg, and 2.0 µg of hGSK-3β plasmid DNA. Un-transfected (UT) cells were a control. Phosphorylated Glycogen Synthase (P-Glycogen Synthase), Beta-Actin as a loading control, Beta-Catenin, and total GSK-3 were probed for by the blot. The intensity of the bands show protein expression levels and the effect of transfecting with hGSK-3β on downstream signaling.

Discussion:

Lithium's effectiveness in mental health treatment has led to tests on its control of dopamine release by neurons, circadian rhythm, neural inflammation, and much more (Chatterjee and Beaulieu, 2022). Our research looks to establish an evidence-backed reason for GSK-3's high Li-sensitivity, hoping to find more precise and effective lithium-based therapies (Chatterjee and Beaulieu, 2022). Separating the effects of Lithium on GSK-3 from lithium on other kinases/sensitive proteins allows us to create Li-resistant proteins that will demonstrate how proteins interact with inhibitors and how they develop resistance to them.

Conclusion:

Between the knowledge of GSK-3's structure that has already been established and the ability to accurately test mutants for lithium resistance, we remain encouraged about the existence of a GSK-3 mutant and will continue to search for it, hoping to further our understanding of its inhibition.

References:

1. Chatterjee, D., & Beaulieu, J. M. (2022, November 24). *Inhibition of glycogen synthase kinase 3 by lithium, a mechanism in search of specificity*. Frontiers in molecular neuroscience. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9731798/
2. Snitow, M. E., Bhansali, R. S., & Klein, P. S. (2021, January 28). *Lithium and therapeutic targeting of GSK-3*. Cells. https://ncbi.nlm.nih.gov/pmc/articles/PMC7910927/