Case Study: Module 4 Small Molecule and Genetic Probes (Part 1)

Methods in Neurobiology

Overview

In this assignment you will describe how to apply the use of small molecule or genetic probes to a specific biological question. This a 20-point assignment.

Instructions

1. Pick a biological question. It can be a new one or it can be the one you presented in module 2/3 and continue with that research topic.
2. Pick a small molecule or genetic probe presented in the material provided or another one that you know about or are interested of.
3. Propose a short research plan (max 1-2 pages without citations) on how you would apply this probe to answer a specific question, what kind of experiment(s) you would do and what you expect to find.
4. Include citations in a format like a scientific publication, especially for the probe selected.

Example One

Question/Biological Problem (10 points)
Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset neurodegenerative disorder characterized by the progressive loss of motor neurons in the brain and spinal cord, which culminates in paralysis and death within a few years of diagnosis. New technologies for gene mapping have enabled the identification of nearly 30 genes in ALS pathogenesis[1]. The first gene discovered associated with familiar forms of ALS is SOD1. To date, over 180 different mutations have been described in SOD1 gene whose function is still unknown. One of the earliest and most common mutations discovered is a glycine to alanine substitution in position 93 of the protein.

Biological Question:
My aim is to investigate the role of SOD1 G93A in the accumulation of toxic oxygen species in cells.

Research Model: To study the function of mutant SOD1, this gene has been stably transfected in human neuroblastoma cell line.

Research Plan (10 points)

- To see how expression of mutant copy of SOD1 affects cell physiology, a genetic probe named HyPer[2] developed to measure intracellular level of H2O2 and therefore production of ROS, would be transiently transfected in the cytoplasm of SOD1 G93A - expressing cells or in a control cell line carrying an empty vector.
- HyPer is based on the fluorescence properties of YFP, whose original sequence has been mutated to be sensitive to ROS accumulation. HyPer has two excitation peaks with maxima at 420 nm and 500 nm, and one emission peak with maximum at 516 nm. Upon exposure to H2O2, the excitation peak at 420nm decreases proportionally to the increase in the peak at 500 nm, allowing ratiometric measurement of H2O2.
- After 2 days cells will be visualized using a confocal laser scanner microscope and images at different wavelengths will be recorded. The decrease of absorption at 420 nm of the probe will be indicative of an increased of intracellular concentration of H2O2.

- If mutant SOD1 impacts the functioning of the redox machinery of the cell directly or by inhibiting endogenous wild-type SOD1, variations in the accumulation of ROS amount will be detected compared to control cells.

1. Beckman JS, Estévez AG, Crow JP, Barbeito L. Superoxide dismutase and the death of motoneurons in ALS. Trends Neurosci. 2001 Nov;24(11 Suppl):S15-20. doi: 10.1016/s0166-2236(00)01981-0. PMID: 11881740
2. HyPer is a product sold by Evrogen. http://evrogen.com/products/HyPer/HyPer_Detailed_description.shtml

Example Two

Question/Biological Problem (10 points)
Recently, the efficacy of antidepressant agents has been a matter of controversy. The selective serotonin reuptake inhibitors (SSRIs), such as citalopram and fluoxetine (Prozac), are currently considered to be a first-line therapeutic tool for the treatment of depression, despite the fact that less than 40% of treated patients respond to this type of antidepressant[1]. SSRIs were thought to moderate depressive symptoms by mainly enhancing the availability of synaptic serotonin (5-HT). However, several findings support the contention that certain SSRIs are able to block other monoamine transporters, such as those implicated in noradrenergic transmission.

Biological Question:
Our aim is to test if fluoxetine, a SSRI that blocks serotonin reuptake, has a similar effect on the noradrenergic system.

Research Model:
Use mouse brain slices from the locus coeruleus (LC).

Research Plan (10 points)
- In order to test if fluoxetine can increase stimulation of LC neurons, a mouse model expressing a noradrenalin optical indicator in LC will be used. Briefly wild-type mice will be injected with a viral vector for targeted expression in LC of GRAB-NE, a genetic probe sensitive to the level of norepinephrin (NE)[2]. Since this probe is specifically sensitive to NE, there is no need to use a specific promoter for selective expression of the probe in a particular type of neuron, since only neurons carrying NE receptors will be activated.
- GRAB-NE is a modular protein composed of an EGFP domain fused to a NE receptor domain able to bind NE. Once this neurotransmitter binds, a rearrangement in the protein causes deprotonation of EGFP and fluorescence emission.
- Mouse brain slices containing LC will be obtained from the mouse line described above and release of NE upon stimulation with fluoxetine or in control conditions will be measure in vivo using laser scanner microscopy.
- If fluoxetine inhibits NE reuptake as it does with serotonin, our method will allow to measure an increase of NE availability at the synapse.

1. Celada P, Puig M, Amargós-Bosch M, Adell A, Artigas F. The therapeutic role of HT1A and 5-HT2A receptors in depression. *J Psychiatry Neurosci*. 2004;29(4):252-265.

2. Feng, J., Zhang, C., Lischinsky, J.E., Jing, M., Zhou, J., Wang, H., Zhang, Y., Dong, A., Wu, Z., Wu, H., Chen, W., Zhang, P., Zou, J., Hires, S.A., Zhu, J.J., Cui, G., Lin, D., Du, J., Li, Y. 2019 A Genetically Encoded Fluorescent Sensor for Rapid and Specific In Vivo Detection of Norepinephrine. Neuron 102(4):745-761.e8.