The Effects of Amyloid Beta on AMPA Receptors

Mia Franco, Dr. Kim Doré, Dr. Robert Malinow
University of California San Diego, San Diego, CA, 9203

Introduction

The AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) subtype excitatory amino acid receptor is responsible for mediating fast synaptic transmission in most CNS (central nervous system) excitatory synapses. When dysfunction occurs in this receptor it can result in mental diseases such as, Alzheimer’s. Thus, the purpose for this study was to observe how amyloid beta (Aβ), a protein that is the main component of amyloid plaques found in the brains of Alzheimer’s patients, affects postsynaptic receptors particularly the AMPA receptor. In order to do this, we introduced the Aβ in neurons with a virus. We used neurons from two kinds or hippocampal preparations. Through the use of fluorescence lifetime imaging (FLIM) we were able to take images of neurons expressing the GluR2 subunit of the AMPA receptor tagged with a GFP (green fluorescent protein) variant that is pH sensitive. More specifically, we were able to measure the effects of Aβ on the fluorescence lifetime of this GFP (SEP) in spines and dendrites.

Alzheimer’s disease

Characterized by:
- Significant neurodegeneration
- Amyloid plaques (comprising amyloid-β)

Aβ comes from the cleavage of the Amyloid Precursor Protein (APP) by 2 enzymes

Methods

Hippocampal preparations used: dissociated cultures and organotypic slices

The hippocampus is important for memory formation and is affected early on in Alzheimer’s patients

Overexpression of SEP-GluR2 to detect endocytosis with fluorescence lifetime imaging (FLIM)

Insulin stimulation induces GluR2 endocytosis

Results

Infection of neurons with a virus expressing CT100 or CTB4 (truncated Aβ)

Here are some images showing infection in red and a transfected neuron in green. By taking a closer look we can measure the fluorescence lifetime in single spines and see how amyloid beta can affect AMPA receptors. To the right, you’ll see a western blot showing infection as well.

PSD-95 blocks GluR2 endocytosis produced by Aβ overexpression

Here, we added PSD-95 to the CT100 and the CTB4 viruses. PSD-95 is a protein that makes synapses stronger. PSD-95 blocks the effect of GluR2 endocytosis produced by Aβ overexpression.

References

H-Y Man et. al, Neuron 2000

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