MITOCHONDRIA AT THE CENTRE OF ALZHEIMER'S DISEASE PATHOGENESIS: ROLE OF NEUROINFLAMMATION

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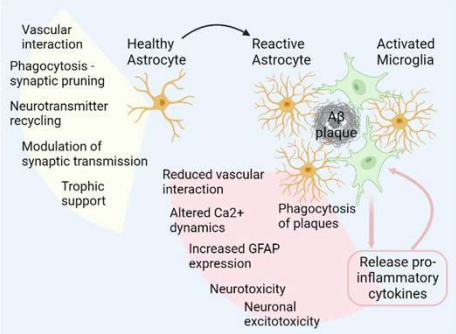
Alzheimer's Disease (AD), a progressive neurodegenerative disease, is the leading cause of dementia worldwide. It is clinically characterised by a progressive loss of memory and cognitive abilities. Histopathological markers include brain region-specific extracellular deposition of Amyloid-β (Aβ) plaques and intracellular Tau neurofibril aggregation (tangles), accompanied by neuronal loss and reduction in brain volume. Currently, disease modifying treatments are few and minimally effective. One major risk factor for developing AD is advancing age, and as the global aging population increases, it is imperative to unravel the molecular mechanisms of AD to identify possible therapeutic targets.

INTRODUCTION

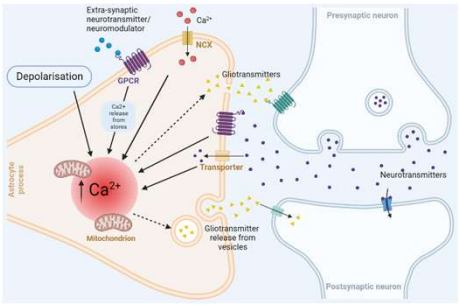
Astrocytes in Alzheimer's Disease Neuroinflammation

Of the non-neuronal cell types of the central nervous system, astrocytes and microglia respond to the presence of Aβ by activating inflammatory responses (1). Traditionally, astrocytes were considered support cells to neurons, but in recent years, their involvement in the inflammatory response and in modulating synaptic transmission have been elaborated.

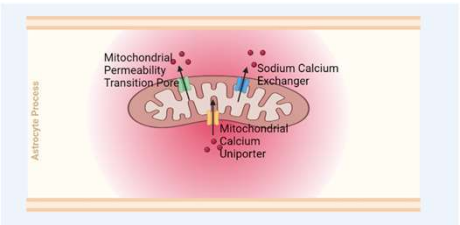


Ca^{2+} signalling in Astrocytes – Involvement of Mitochondria

Astrocytes possess several receptors that respond to extracellular stimuli through increases in intracellular Ca^{2+} levels. These Ca^{2+} elevations have been linked to several physiological functions of astrocytes, including modulation of synaptic transmission (3).



Astrocytic mitochondria localize to the regions of Ca^{2+} elevation and, by actively taking up or releasing Ca^{2+}, tune the amplitude, duration and propagation of the Ca^{2+} signal (4)



AIM

To investigate the role of mitochondrial Ca^{2+} handling and bioenergetics in astrocyte activation during Alzheimer's disease linked neuroinflammation, in order to identify potential targets for therapeutic interventions.

METHODS

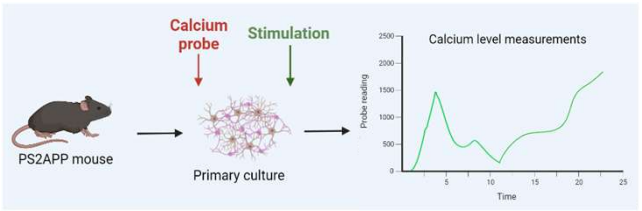
Mouse model of Alzheimer's Disease

To model AD, we rely on disease-associated mutations in 2 genes implicated in early onset (familial) AD: the gene for amyloid precursor protein (APP) and the gene for Presenilin 2 (PSEN2), which is involved in the cleavage of APP leading to the formation of Aβ.

The PS2APP line used here is generated by introducing human APP with "Swedish" mutation (K670N, M671L) and PSEN2 with the single mutation N141I into pure background mice (C57BL6, which is used as Wild Type for comparison)

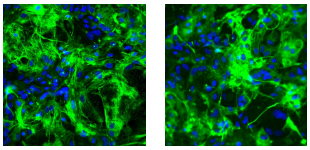
Ca^{2+} Measurements

Either, proteins that produce fluorescence when bound to Ca^{2+} are genetically encoded in cell culture or, fluorescent molecules that produce a shift in signal upon Ca^{2+} binding are loaded into the cells. Under a fluorescence microscope, astrocytes in culture are stimulated by the addition of molecules that cause a Ca^{2+} response.



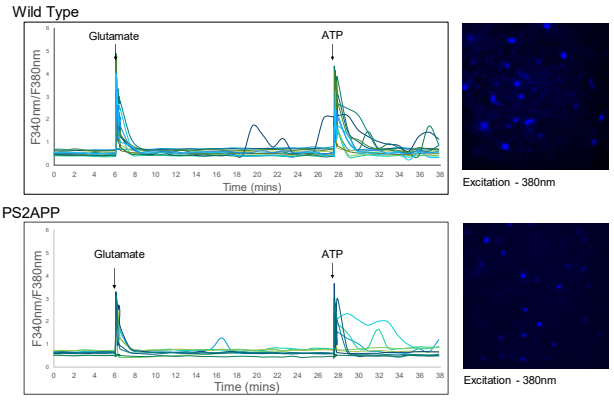
RESULTS

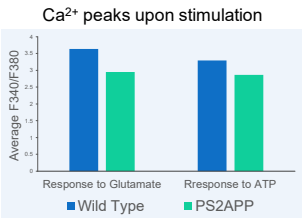
Pure cortical astrocytes cultured from neonatal PS2APP and Wild Type mice, loaded with a cytosolic Ca^{2+} binding fluorescent molecule FURA-2, which emits light at 510nm. Excitation is possible with light of 340nm and 380nm wavelengths and the ratio of the emissions is used to estimate Ca^{2+} levels within the cell. The cells are stimulated with glutamate (a neurotransmitter) and ATP (a neuromodulator).



WT PS2APP

Astrocytes in the pure cultures were characterized by immunostaining with anti-GFAP antibody [green] (nuclei seen with Hoechst stain [blue])





PS2APP astrocytes have lower peaks of cytosolic Ca^{2+} upon stimulation compared to Wild Type cells.

FURTHER STEPS

Once these preliminary results are confirmed by more trials, the next step is to utilise genetically encoded, mitochondria targeted Ca^{2+} probes to analyse whether these differences are also reflected in mitochondrial Ca^{2+} dynamics of astrocytes. Further investigations into inflammatory pathways will be carried out to elucidate how changes in Ca^{2+} signalling affect the functioning of reactive astrocytes in the AD model.

REFERENCES

(1) Meraz-Ríos, M. A., et al, Frontiers in Integrative Neuroscience (2013)
(2) Spanos, F., & Liddelow, S. A., Cells (2020)
(3) Semyanov, A., Henneberger, C., Agarwal, A, Nature Reviews Neuroscience, (2020).
(4) Jackson, J. G., & Robinson, M. B., The Journal of Neuroscience (2015).

- All schematics were created with BioRender.com

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