Author: Aleksandra Tracichleb
Supervisor: Dr Gayle Doherty
School of Psychology and Neuroscience
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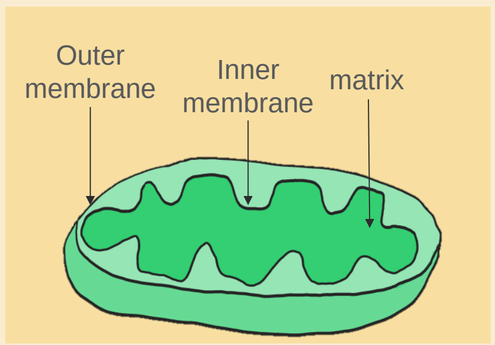
University of St Andrews

Serum starvation in HT-22 mouse hippocampal cells leads to the hyperpolarization of the mitochondrial membrane potential

01 Definitions

- **Mitochondria**- organelle responsible for aerobic respiration, supply cell with energy
- **Δψm**- mitochondrial membrane potential, the electrical difference between the mitochondrial matrix & cytosol (Aldana et al. 2021)
- **JC-1**- Fluorescent dye assessing the Δψm. It emits red fluorescence in healthy mitochondria and green fluorescence in the unhealthy ones (Sivandzade et al., 2019)
- **Leptin**- hormone involved in regulation of food intake and fat storage. It was shown to be neuroprotective (Cheng et al. 2020)



02 Introduction

Mitochondrial function is crucial for neurons due to their high energy demand (Allen et al., 2018). Recent research has revealed as the mitochondrial impairment might be an underlying cause of diseases such as Alzheimer's and Parkinson's. This study aimed at investigating the effects of serum withdrawal on the Δψm in HT-22 hippocampal mice cells. Moreover, it was aimed at investigating the effect of enriched and standard environments on Mfn1 and Fis1 fission and fusion proteins in rats hippocampi.

03 Methods & Analysis

JC-1 assays:

- 4 groups of HT-22 cells were present: G- with serum retained, F with no serum, E with no serum and 1nM of leptin and D with no serum and 10nM of leptin
- Red and green fluorescence were read after 2, 4 and 6 hours from the serum withdrawal.
- Red-to-green fluorescence ratio was used to assess Δψm.

Bradford assays:

- Used to assess initial protein levels in all brain samples.
- 10-fold dilution with BSA was done to create a normal curve.
- 10µl of the sample was mixed with 10µl of 1M NaOH and 500µl of Bradfordt reagent
- The absorbance was read at 570nm.

ELISA assays:

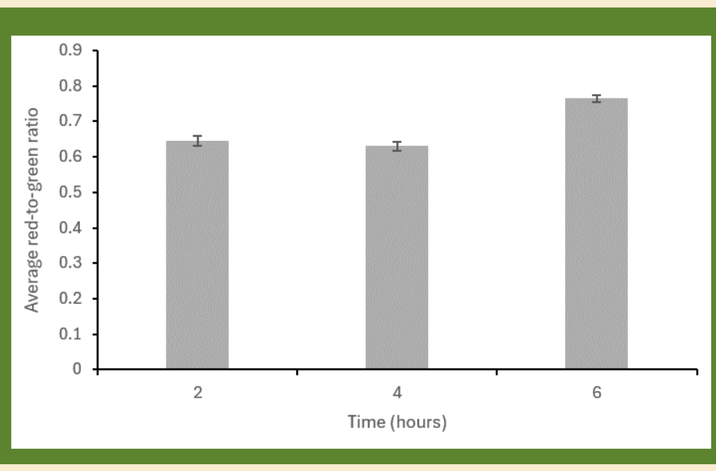
- Primary antibodies Mnf1, Fis1 and alpha tubulin and secondary anti-mouse and anti-rabbit antibodies were used.
- ELISA assays were used to establish the concentration of fission and fusion proteins.

Analysis:

- One-way ANOVA was used. $p < 0.05$ was considered as significant.

04 Results

The graph displays the average red-to-green fluorescence ratio against the time of the serum withdrawal duration. The red-to-green ratio is significantly increased for 6-hour condition compared to 2 and 4-hour conditions. Therefore, the Δψm was significantly higher 6 hours after the serum withdrawal.



Main findings

01

The mitochondrial membrane potential was significantly higher 6 hours after the serum withdrawal compared to 2 and 4 hours of serum withdrawal.

02

There was no significant effect of leptin in 2, 4 and 6 hour serum withdrawal conditions.

05 Discussion & Future Research

- Study by Ijima et al. has shown that hyperpolarisation of Δψm can be an intermediate stage in apoptosis, with nutrient-deprived cells also showing cytochrome c release (2003). In contrast, Colombaioni et al. shown that serum-deprived cells displayed activation of apoptotic pathway without any changes in Δψm (2002).
- Secondly, the lack of effect of leptin didn't confirm the findings in the literature. Chang et al. have demomnstarted that leptin exerts its neuroprotective effects through regulation of fission and fusion proteins and inhibition of Δψm decrease. The observed differences in leptin effects might have been due to the concentration used- low concentrations, of 0.1nM, compared to 1nM used in this study.
- The results from Bradford and ELISA were not obtained due to the lack of protease inhibitor in homogenized rats brain tissues. This restulted in protein degradation.. In future studies it should be ensured that appropriate protease inhibitor is added to obtain accurate Bradford and ELISA values.
- Future research should investigate mitochondrial bioenergetics' role in neurodegenerative diseases. For instance, it was established that Aβ interacts with fission and fusion proteins in Alzheimer's, however, the direction of the causation still remains unknown (Wang *et al*., 2009). Hence, research exploring the effects of mtDNA mutations of the development of neurodegenerative disorders should be expored to unravel their cause and offer new therapeutic targets

06 Bibliography

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