Effect of Hyperglycemia on Autophagy in Neurons

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Introduction

- Autophagy is a cellular mechanism that degrades and reallocates protein aggregates (**Figure 1**). Autophagy dysfunction results in toxic protein accumulation, which is found in the development of hyperglycemia.[1]
- Light chain protein 3B (LC3B) is an autophagy marker. LC3B exists in two forms, LC3B-I and LC3B-II. During autophagy, LC3B-I is converted to LC3B-II, so LC3B-II expression increases.[2]
- Sequestosome 1 (SQSTM1/p62) is another autophagy marker. During autophagy, P62 is degraded and decreases in expression.[3]
- The objective of this work is to understand autophagy in the context of hyperglycemia (as found in diabetes, e.g. 50mM glucose).

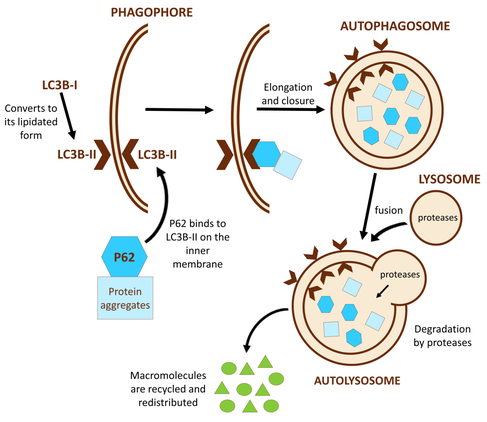

Figure 1. Illustration of the autophagy mechanism of a cell.

Materials and Methods

- Human neuroblastoma SH-SY5Y clonal cell line and adult rat dorsal root ganglia (DRG) primary sensory neurons were cultured in low (5-10mM) and high (50mM) concentrations of D-glucose.
- To analyze autophagic flux, SH-SY5Y cells and DRG neurons were treated with an autophagy inhibitor, bafilomycin A1 (Baf A1).
- LC3B-I, LC3B-II and p62 expression were analyzed by semi-quantitative Western blotting.[4]

Results and Discussion

Autophagy in human neuroblastoma SH-SY5Y cells

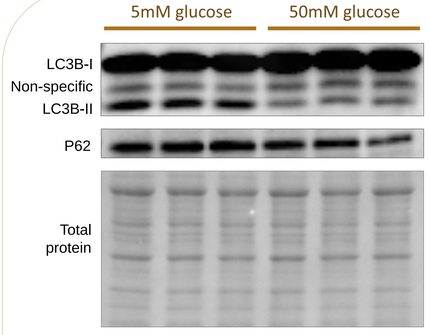

Figure 2. Western blots for LC3B and p62. SH-SY5Y cells were cultured for 48h under the control (5mM glucose) or the treatment (50mM glucose) condition; n=3 replicate cultures.

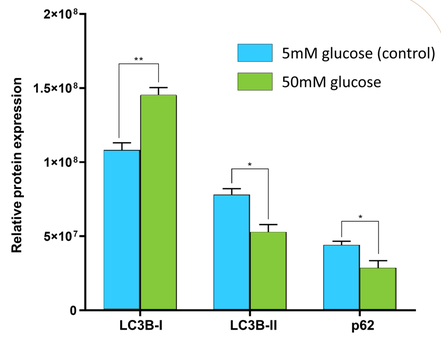

Figure 3. The relative protein expression of LC3B-I, LC3B-II and p62. Data presented is from total protein normalization. Data reported as the mean of n=3 with the standard of error of mean (SEM). Unpaired t tests were applied (*p ≤ 0.05, **p ≤ 0.01).

- Western blots for LC3B were validated using 2 antibodies. The top band was LC3B-I, the middle band was non-specific binding, and the bottom band was derived from LC3B-II (**Figure 2**).
- Under hyperglycemic conditions, LC3B-II expression decreased significantly, indicating that autophagy is being inhibited (**Figure 3**).
- However, under hyperglycemic conditions, p62 expression decreased unexpectedly (**Figure 3**).

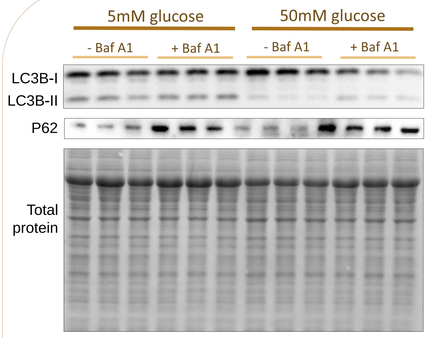

Figure 4. SH-SY5Y cells were cultured for 72h with low or high glucose, in the presence or absence of bafilomycin A1 for the last 2h; n=3 replicate cultures.

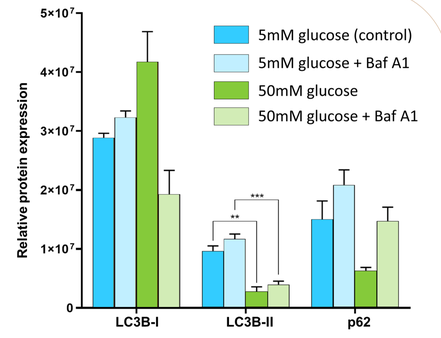

Figure 5. The relative protein expression of LC3B-I, LC3B-II and p62. Data presented is from total protein normalization. Values are the mean +/- SEM, n=3. One-way ANOVA with Tukey's multiple comparison post-hoc test (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).

- Similar results were observed in the changes of LC3-II and p62 expression from low glucose to high glucose concentration (**Figure 3 and 5**).
- The unexpected decrease of p62 expression under hyperglycemic conditions (**Figure 3 and 5)** might be because p62 levels take a longer time to change compared to LC3B.[4] It may also be related to the p62 distribution to an insoluble compartment of the cell.[4]
- All cells treated with Baf A1 increased expression of LC3B-II and p62, indicating the autophagic degradation of proteins was successfully blocked (**Figure 5**).

Autophagy in cultured adult DRG neurons

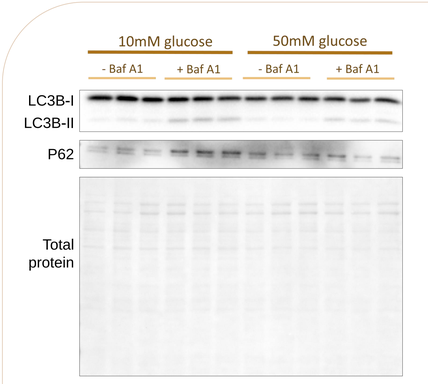

Figure 6. DRG neurons were cultured for 72h with low or high glucose, in the presence or absence of bafilomycin A1 for the last 2h; n=3 replicate cultures.

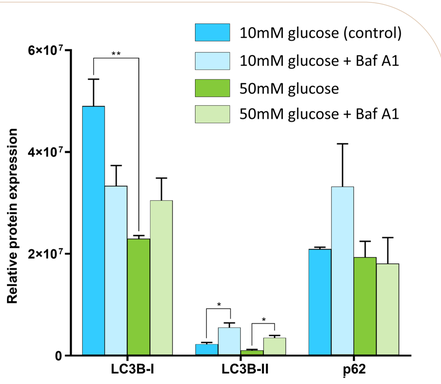

Figure 7. The relative protein expression of LC3B-I, LC3B-II and p62. Data presented is from total protein normalization. Values are the mean +/- SEM, n=3. One-way ANOVA with Tukey's multiple comparison post-hoc test (*p ≤ 0.05, **p ≤ 0.01).

- Similar to the human SH-SY5Y cells, LC3B-II expression in rat DRG neurons decreased under high glucose (**Figure 7**)**.**
- Unlike the human SH-SY5Y cells, p62 expression from DRG neurons did not vary much between treatment groups.
- In all cells treated with Baf A1, LC3B-II expression increased significantly, indicating that autophagy was successfully blocked.

Conclusions

- Based on LC3B-II results, under hyperglycemic conditions, autophagy was inhibited in both human SH-SY5Y cells and rat DRG primary sensory neurons.
- The treatment of Baf A1 successfully blocked autophagic flux. In DRG under hyperglycemic conditions, autophagic flux, although inhibited, remained present.
- The unexpected decrease of p62 expression under hyperglycemic conditions might be due to the fact that p62 levels take a longer time to change compared to LC3B.[4] P62 may also be distributed to an insoluble compartment of the cell.[4] Further work should be conducted with sample pellets.

References

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Acknowledgements

I would like to thank the University of Manitoba for supporting me with the Undergraduate Research Award. I would also like to express my gratitude to Dr. Farhana Naznin and Dr. Paul Fernyhough for their guidance on this project. Finally, I would like to thank all the members of the Fernyhough laboratory for their support.