1. Introduction

What is L-Ergothioneine (ET)?

L-Ergothioneine (ET) is a naturally occurring thiol derivative of histidine with unique properties: 

- Highly stable at physiological pH
- Not endogenously produced within the human body, entirely taken up via diet
- Presence of a highly specific endogenous transporter, OCTN1, within the human body
- Cell impermeable
- Relatively slow excretion

Presently, its physiological function remains unknown.

Proposed Mechanism of Action

Previously, ET has been suggested to have antioxidant effects such as: 

- Scavenger of •OH radicals, HOCI and GNO(2)(-) 
- Chelator of divalent metal cations 
- Protection from UV radiation damage

ET's relationship to Mitochondria

ET has been shown to contribute to mitochondrial function by: 

- Protection against mDNA damage by HOCI 
- Transfected GFP-OCTN1 was identified on mitochondrial membranes

2. Objectives

A. To assess ET uptake into the mitochondria
B. To identify how ET interacts with the mitochondria
C. To explore if ET confers any beneficial effect relative to its effect within the mitochondria
D. To identify if ET has therapeutic potential in age-associated diseases involving mitochondrial dysfunction

3. Methods

ET's Uptake into the Mitochondria

- Increasing levels of ET detected within mitochondria after in vitro treatment
- Virtually undetectable levels in OCTN1−/− mice with no increase after feeding

ET's Interaction with Electron Transport Chain

- ET's interaction with each Mitochondrial Complex was examined in isolation in this assay
- Heart mitochondria were incubated with respective complex inhibitors and ET
- Complex substrate was then added together with assay reagents
- Complex activity was then determined with microparticle reader by measuring rate of change of substrate

ET's Effect on Cellular Health

- ET-containing medium was used and Rotenone was added for 24h
- Rotenone was removed and the appropriate assay reagents were added
- Final measurements were done with a microparticle reader or flow cytometer depending on the assay

4. Results and Discussion

ET's Uptake into the Mitochondria

- MTTS/ATP assay

ET's Interaction with Electron Transport Chain

- 1h ET Pre-treatment on cell

ET's Effect on Cellular Health

- SH-SYSY cells were seeded into 96-well plates and cells were incubated with ET for one hour
- ET-containing medium was used and Rotenone was added for 24h
- Rotenone was removed and the appropriate assay reagents were added
- Final measurements were done with a microparticle reader or flow cytometer depending on the assay

5. Conclusions

A. ET is very likely to be taken up into the mitochondria after entering the cell, and this uptake is likely mediated through OCTN1.
B. While its endogenous function remains unknown, cells exposed to ET had lower levels of mitochondrial ROS as compared to control.
C. ET's interaction with Complex I inhibitors and Complex V is yet to be examined in greater detail as it may point towards ET's potential endogenous function.
D. Further work is needed to elucidate ET's therapeutic potential within the mitochondria but our present findings suggest that ET has promise as a mitochondria-targeted antioxidant.

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7. References