# Rotenone induces alterations to lipid and glutamine utilization in SH-SY5Y cells

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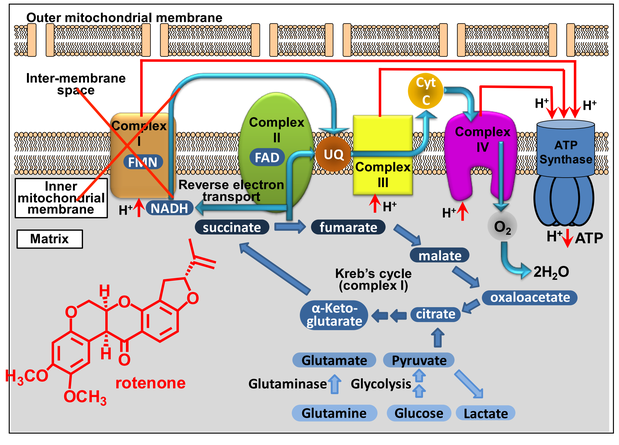
## Abstract

Rotenone is a naturally occurring mitochondrial complex I inhibitor with a known association to Parkinsonian phenotypes in both human populations and rodent models. Despite this finding, a clear mechanistic link between rotenone exposure and neuronal damage remains to be determined. Here, we report alterations to lipid and glutamine metabolism in SH-SY5Y cells exposed to rotenone. The intracellular concentrations of acetyl-coenzyme A (CoA) were found to be maintained in spite of a significant decrease in glucose derived acetyl-CoA (1). Furthermore, palmitoyl-CoA levels were maintained, whereas, levels of many of the medium chain acyl-Coenzyme A species were significantly reduced. Additionally, using isotopolog analysis it was found that β-oxidation of fatty acids of varying chain lengths helped maintain acetyl-CoA levels. Rotenone also induced increased glutamine utilization for lipogenesis, in part through reductive carboxylation. Taken together, these findings show alterations to lipid and glutamine metabolism play an important compensatory role in response to complex I inhibition by rotenone.

## Introduction

Epidemiological studies have suggested a positive correlation between rotenone exposure and Parkinson's Disease (PD) in human populations. Despite a known association between rotenone and dopaminergic neuronal damage, the mechanistic cause of neuronal cell death remains unknown. There is substantial evidence to suggest that mitochondrial dysfunction plays an important role in the development of PD.

Therefore, defects in mitochondrial metabolism could be important to the pathogenesis of PD. Here we report compensatory metabolic alterations to lipid and glutamine metabolism in response to complex I inhibition by rotenone in SH-SY5Y cells.




**Rotenone inhibits mitochondrial complex I**

## Methods

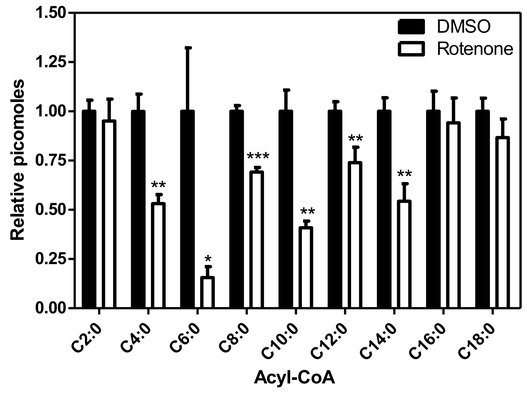
Extraction and LC-MS analysis of acyl-CoAs has been described in detail previously. (2,3) A novel method using *N-tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA), a common derivatization reagent for GC-MS, has been converted to allow for the analysis of the derivatives of citrate, isocitrate, and alpha ketoglutarate on a C18 reversed phase LC-MS. Derivatization with pentafluororbenzyl bromide (PFBBr) coupled to liquid chromatography-electron capture atmospheric pressure chemical ionization/mass spectrometry (LC-ECAPCI/MS) allows for the analysis of succinate, fumarate, and malate via normal phase LC-MS.

**Liquid chromatography- mass spectrometry**

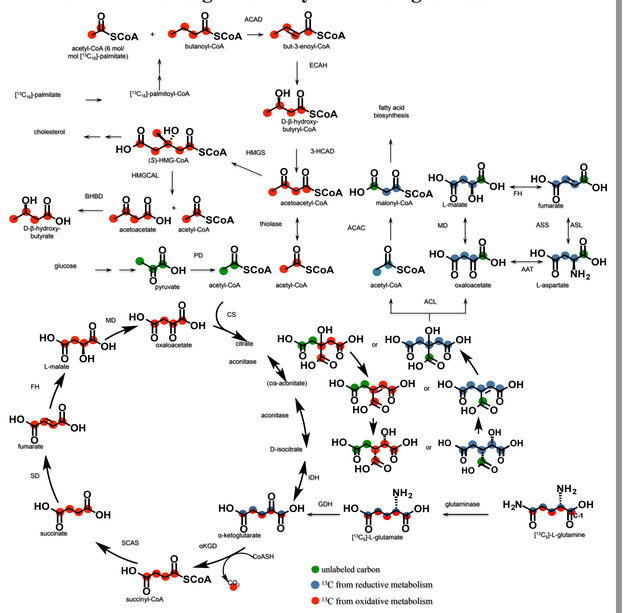
- The tBDMS derivatives of citrate, isocitrate and α-ketoglutarate were separated using a reversed-phase HPLC Xbridge BEH130 C18 column (2.0 mm × 50 mm, pore size 3.5 µm) with 5 mM ammonium acetate in water/ACN/formic acid (40:60:0.1; v/v/v) as solvent A, and ACN/ formic acid (100:0.1; v/v) as solvent B at a flow rate of 500 µL/min. Samples were analyzed using an API 4000 triple-quadrupole MS equipped with an electrospray source operating in positive ionization mode.
- Succinate, fumarate, and malate were separated using a Chiralpak AD-H column (250 × 4.6 mm i.d., 5 µm; Daicel Chemical Industries, Ltd., Tokyo, Japan) at a flow rate of 1 mL/min. Solvent A was hexanes and solvent B was IPA/methanol (1:1; v/v). MS analysis was conducted on a Thermo Finnigan TSQ Quantum Ultra AM mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source in the electron capture (EC) negative ion mode.

## Results

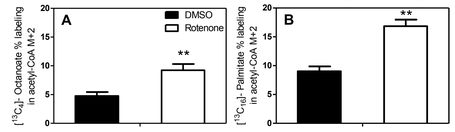
- **Rotenone decreases medium chain acyl-CoA species**




- **Carbon labeling from fatty acids and glutamine**

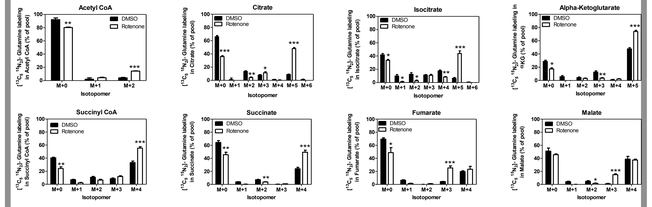



- **β-oxidation of fatty acids supports acetyl-CoA production**



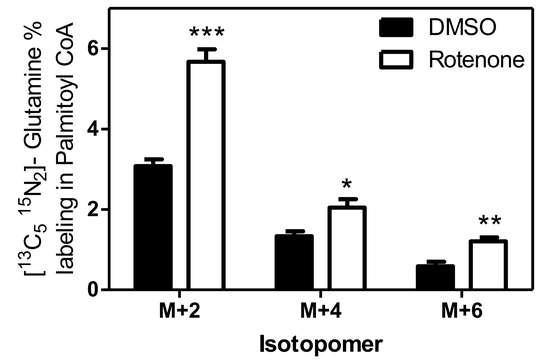

SH-SY5Y cells were treated with 100 µM [$^{13}C_4$]- octanoate (A) or [$^{13}C_{16}$]-palmitate (B) and either 100 nM rotenone or vehicle control DMSO for 6 hours.

- **Rotenone induces reductive glutamine metabolism**




SH-SY5Y cells were treated with 2 mM [$^{13}C_5$ $^{15}N_2$]-glutamine and either 100 nM rotenone or vehicle control DMSO for 6 hours. Rotenone induces reductive glutamine metabolism by the action of isocitrate dehydrogenase (IDH). Notably, rotenone treatment results in a significant enrichment in the M+5 isotope of citrate and isocitrate. Additionally, citrate derived from reductive carboxylation undergoes cleavage by ATP citrate-lyase (ACL), yielding an enrichment in the M+3 isotope of fumarate and malate.

- **Reductive glutamine metabolism contributes to lipogenesis**




SH-SY5Y cells were treated with 2 mM [$^{13}C_5$ $^{15}N_2$]-glutamine and either 100 nM rotenone or vehicle control DMSO for 6 hours. Isotopic enrichment in palmitoyl-CoA indicates an increase in glutaminolysis sopports lipogenesis in response to rotenone.

## Conclusion and future directions

- Rotenone induces compensatory metabolic alterations to maintain acetyl- and palmitoyl-CoA levels while medium chain acyl-CoAs decrease
- Oxidation of multiple fatty acids for acetyl-CoA production is upregulated in response to complex I inhibition by rotenone
- Glutamine supports both short and long acyl-CoA levels in part through reductive carboxylation in rotenone treated cells
- Other metabolic substrates may play an important compensatory roles
- Primary rat cortical neurons will provide a better model for studying the pathogenesis of PD
- Neuronal damage caused by rotenone may be due in part to previously unidentified metabolic abnormalities

## References

(1) Basu SS.; Blair IA. Rotenone-mediated changes in intracellular coenzyme A thioester levels: implications for mitochondrial dysfunction. Chemical Research in Toxicology. (2011)

(2) Snyder NW.; Basu SS.; Zhou Z.; Worth AJ.; Blair IA. Stable isotope dilution liquid chromatography-mass spectrometry analysis of cellular and tissue medium- and long-chain acyl-coenzyme A thioesters. Rapid Communications in Mass Spectrometry. (In press)

(3) Basu SS.; Blair IA. SILEC: a protocol for generating and using isotopically labeled coenzyme A mass spectrometry standards. Nature Protocols. (2011)

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