

Decreased Respiratory Capacity and Glycolytic Rates in a Rat Model of Temporal Lobe Epilepsy Revealed by Metabolic Flux Analysis of Hippocampal Synaptosomes

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Abstract

Metabolic dysfunction is emerging as an important mechanism in the pathogenesis of temporal lobe epilepsy (TLE). TLE is the most common form of acquired epilepsy in which injury leads to epilepsy development by a process known as epileptogenesis. Epileptogenesis is the process whereby a precipitating injury activates a series of cellular and molecular changes that ultimately lead to the development of a hyperexcitable state in which spontaneous recurrent seizures are observed. To determine the relative contributions of mitochondrial respiration and glycolysis during kainate-induced epileptogenesis, real-time analysis of oxygen consumption and extracellular acidification were assessed in isolated synaptosomes from the hippocampus of rats at various times (3h, 8h, 16h, 48h, 7d, and 3 wk) after kainate administration using an extracellular flux analyzer (Seahorse Bioscience, North Billerica, MA, USA). Overall respiratory capacity, ATP-linked respiration and spare respiratory capacity were decreased at the 16h and 48h time-points and returned to control levels at the 7d time-point. After 3 weeks the deficits return. Baseline rates of glycolysis were unchanged compared to controls. However, treatment with the mitochondrial uncoupler FCCP and ATP synthase inhibitor oligomycin resulted in a time-dependent decrease in glycolytic rates which recovered at the 7d time-point in kainate treated rats. These data suggest that status epilepticus produces a time-dependent deficit in mitochondrial oxygen consumption and glycolytic rates which coincide with increases in indices of mitochondrial oxidative stress previously shown in the laboratory. This study provides further evidence of metabolic dysfunction in experimental TLE.

Goals

Hypothesis: It is hypothesized that mitochondrial and glycolytic metabolism are impaired in experimental temporal lobe epilepsy.

Goal: To simultaneously monitor respiration and glycolysis in a rat model of temporal lobe epilepsy using an extracellular flux analyzer.

Methods

Kainate Treatment

Sprague Dawley rats (~300-350g) were administered kainate (KA) dissolved in sterile saline pH 7.4 or vehicle (Control-Ctl) subcutaneously and observed for seizure activity. Within ~30 min after kainate injection, rats develop status epilepticus which subsides after 3-4 hours. Hippocampal tissue was harvested at 3, 8, 16, 48 hours, 7 days, and 3 weeks for extracellular flux analysis on the XF24 Analyzer (see below).

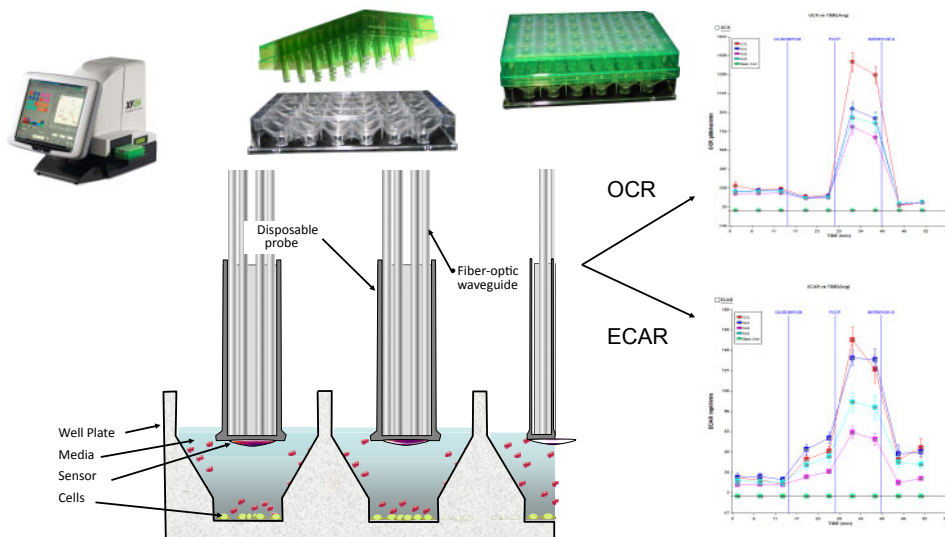
Isolation of Hippocampal Synaptosomes

Tissue was homogenized using a dounce tissue grinder (Wheaton, Millville, NJ) in 5-10% w/v (50-100mg tissue/ml) homogenizing buffer. The homogenate was processed as in Choi et al with some modifications.

Plating Synaptosomes in Seahorse Cell Culture Plates

Synaptosomes are re-suspended in an ionic buffer and diluted to a .2 mg/ml solution. 50 µl is then aliquot into each well of a PEI-coated Seahorse plate and spun down at 2300 g for 1 hour at 4°C. After this spin, the ionic buffer is carefully removed and replaced with an incubation buffer. The plate is then run on the XF24 Analyzer (explained below) to obtain oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) indicating mitochondrial respiration and glycolytic rates respectively.

Seahorse XF24 Analyzer



Images courtesy of: Seahorse Bioscience

Background

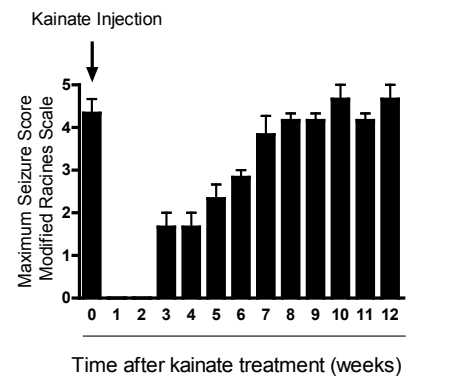
Development of the Kainate Model

Kainate injection leads to status epilepticus

A seizure free "latent period" is observed up to 3 weeks

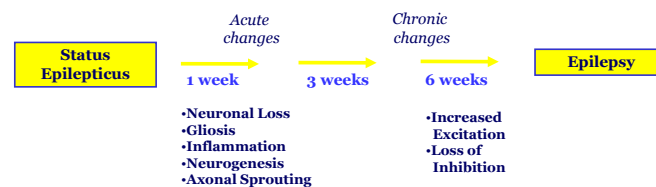
Seizure severity increases after 3 weeks

Seizure frequency increases after 3 weeks

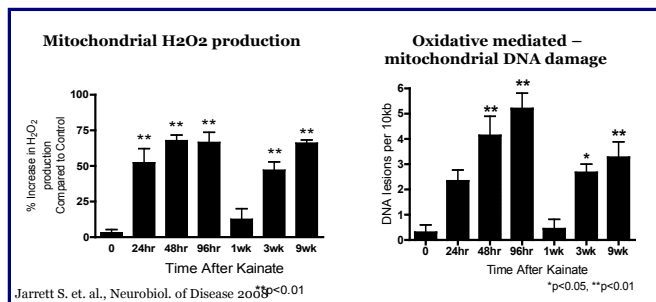


Jarrett S. et. al., Neurobiol. of Disease 2008

Epileptogenesis in the Kainate Model of Epilepsy



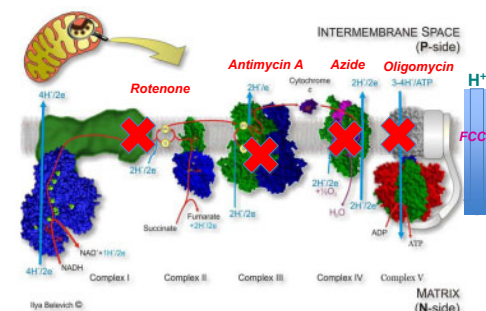
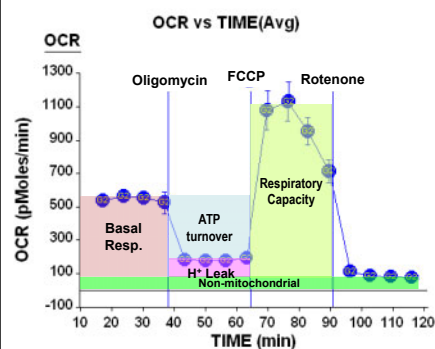
Previous Work Indicating Oxidative Stress in Epileptogenesis



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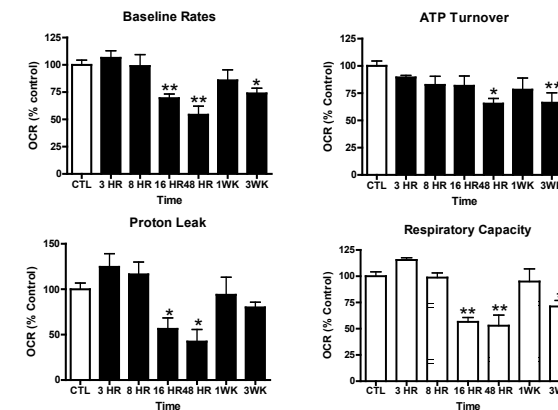
Experimental Design and Compounds

Experiment to Analyze Mitochondrial Respiration

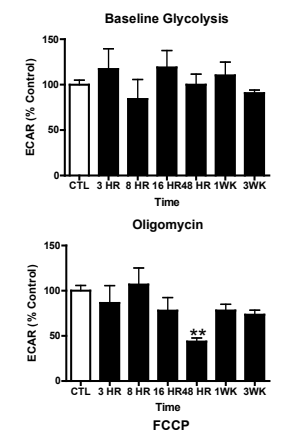


Results

Mitochondrial Respiration



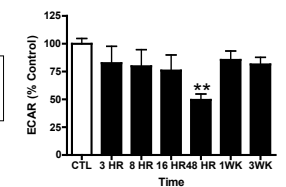
Glycolysis



Characterization of Synaptosomal Fraction

Table with 3 columns: Mitochondria (COX IV), Glial (GLT1, IBA1, GFAP), and Synaptosomal (SNAP-25).

*= p<.05
**= p<.01



Mitochondrial Respiration

- At 16 and 48 hours, baseline OCR, ATP turnover, proton leak, and respiratory capacity are decreased in KA-treated animals compared to control.
• This recovers at the 7-day time point and returns to decreased values at 3 weeks.
• Deficits in mitochondrial respiration shown here coincide with mitochondrial oxidative stress shown previously in the lab.

Glycolysis

- Similar to mitochondrial deficits, at 48 hours oligomycin and FCCP stimulated glycolysis is decreased in KA-treated animals.
• This deficit recovers at the 7 day time point but does not return at 3 weeks.

Summary

- Mitochondrial deficits shown above are indicative of metabolic dysfunction during epileptogenesis which may contribute to cell loss and/or damage.
• Stimulating the synaptosomes with oligomycin and FCCP shows a defect in glycolytic rates which indicates possible damage to glycolytic enzymes during epileptogenesis.

Future Directions

- Previous work in the lab has shown that at 6 weeks after KA treatment, indices of mitochondrial oxidative stress increase after the recovery seen at 7 days. Therefore, these same time points would be ideal to test if mitochondrial and glycolytic deficits persist.

Acknowledgements

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Synaptosome isolation method was adapted from Choi et al., 2009 and Dunkley et al., 2008.

Choi SW, Gerencser AA, Nicholls DG. Bioenergetic analysis of isolated cerebrocortical nerve terminals on a microgram scale: spare respiratory capacity and stochastic mitochondrial failure. J Neurochem. 2009. May;109(4):1179-91.

Dunkley PR, Jarvie PE, Robinson PJ. A rapid Percoll gradient procedure for preparation of synaptosomes. Nat Protoc. 2008;3(11):1718-28.

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