Glycolytic and Redox Control of Neuronal Excitability *In Vitro*

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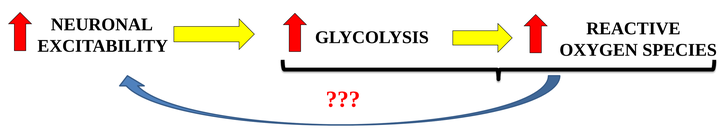
Abstract

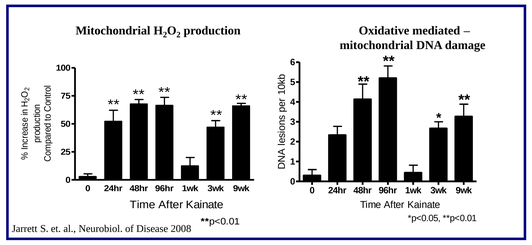
Metabolic control of epilepsy is recognized in part due to the efficacy of ketogenic diets, which provide alternate fuels rather than glucose for neuronal activity. It is documented that glycolytic rates are acutely increased during epileptic seizures and inhibition of glycolysis with 2-deoxyglucose (2-DG) has anti-seizure effects. Work from our laboratory has shown that seizure activity increases the steady-state levels of reactive oxygen species (ROS) production and causes mitochondrial dysfunction. Here we determined the relationship between glycolysis, ROS production and neuronal excitability. Mixed rat primary cortical cultures treated with 4-Aminopyridine (4-AP), a potassium channel blocker, showed an increase in a) Extracellular Acidification Rate (ECAR) levels (measure of glycolytic rate in the Seahorse XF analyzer), b) ROS production, measured by Amplex Red and c) increased neuronal excitability, assessed by a multiple electrode array system (Axion Biosystems). Pre-treatment of mixed primary cortical cultures with compounds like 2-DG, Bromopyruvic acid (3BP), palmitate - an anaplerotic substrate (along with glycogenic substrate limitation), nicotinamide riboside (NR) – an NAD^+ precursor, oxaloacetate (OA) - a TCA cycle intermediate, and mTOR inhibitor rapamycin decreased ECAR rates and neuronal hyper-excitability induced by 4-AP. Pre-treatment with Thiazolidinediones – an acute specific inhibitor of the mitochondrial pyruvate carrier, PI3K inhibitor Wortmannin decreased 4-AP induced increase in glycolysis, without significantly altering neuronal hyper-excitability. Lastly, 2-DG also reversed ROS production produced by 4-AP. Taken together, these results suggest that glycolysis contributes to ROS production, which in turn increases neuronal hyper-excitability.

Hypothesis



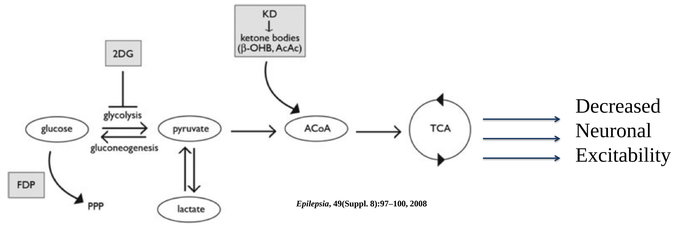
Background

Previous Work Indicating Oxidative Stress in Epileptogenesis



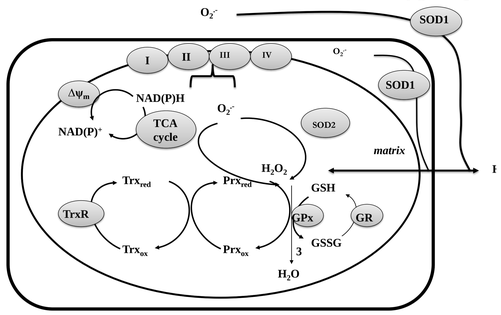
Jarrett S. et. al., Neurobiol. of Disease 2008

Glycolytic Modulation of Neuronal Activity through 2-DG and Ketogenic Diet

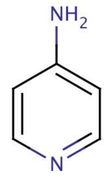


Epilepsia, 49(Suppl. 8):97–100, 2008

Production and Detoxification of Reactive Oxygen Species



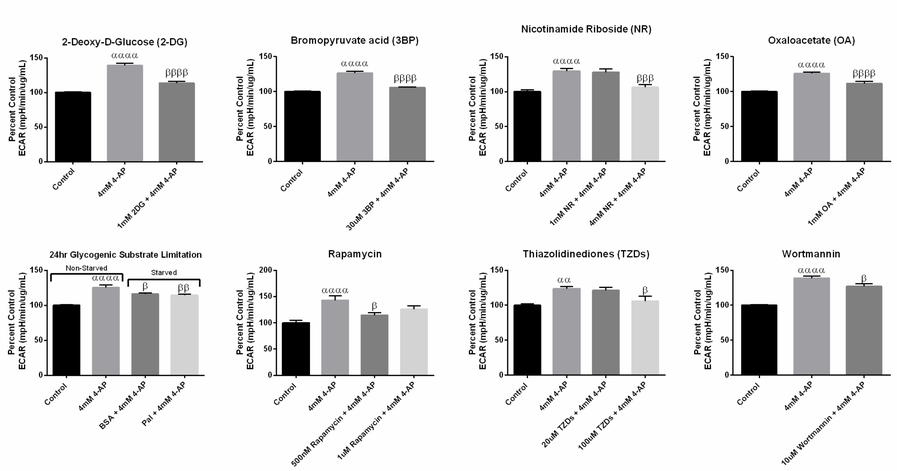
Potassium Channel Blockers to Induce Neuronal Excitability



4-Aminopyridine

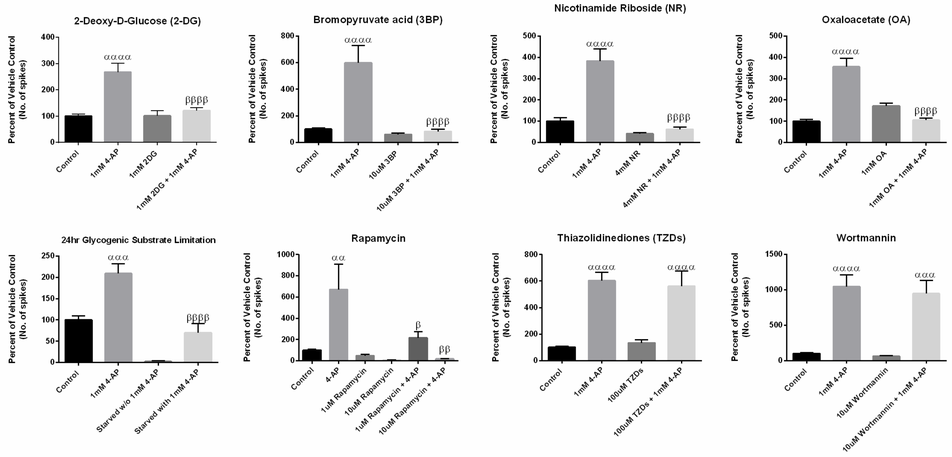
Results

Pharmacological Inhibition of 4-AP-induced Glycolytic Rates



Figures above describe the changes in glycolytic rates after administering 4-AP when pre-treated with different pharmacological inhibitors.
α represents values compared to control group and β represents values compared to 4mM 4-AP group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Effect of Bioenergetics Modulators on 4-AP-Induced Neuronal Excitability



Figures above depict the changes in neuronal excitability after adding 4-AP when pre-treated with different pharmacological inhibitors. .
α represents values compared to control group and β represents values compared to 1mM 4-AP group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

2-Deoxy-D-glucose Attenuates 4-AP Induced ROS Production

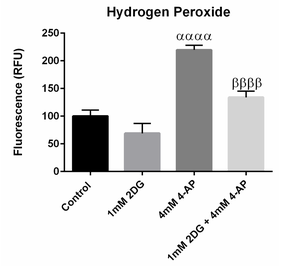
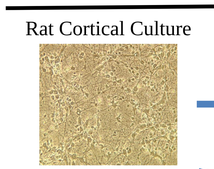
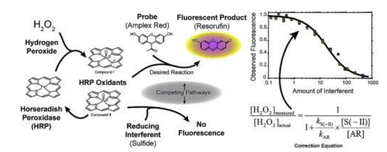


Figure shows the a) increase in hydrogen peroxide levels induced by 4AP and b) the attenuation when pre-treated with 2-DG. α represents values compared to control group and β represents values compared to 4mM 4-AP group. , ****p<0.0001.

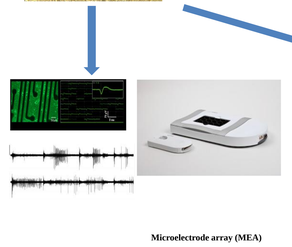
Methods

Rat Cortical Culture

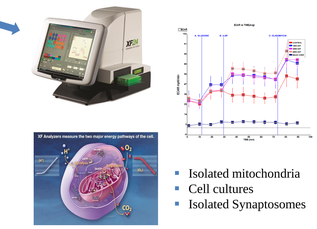




Wang N. et al, Analytical Chimica Acta 2017



Microelectrode array (MEA)



- Isolated mitochondria
- Cell cultures
- Isolated Synaptosomes

Summary and Conclusions

- Increasing neuronal excitability with 4-AP causes an increase in glycolysis (ECAR) and hydrogen peroxide levels.
- Inhibition of glycolysis with 2-DG, 3BP, 24 hours glycogenic substrate limitation, nicotinamide riboside (NR), oxaloacetate, rapamycin significantly inhibit ECAR and neuronal excitability.
- Pre-treatment with an acute specific inhibitor of the mitochondrial pyruvate carrier-Thiazolidinediones, Wortmannin decreased 4-AP induced increase in glycolysis, without significantly altering neuronal hyper-excitability.
- 2-DG attenuates 4-AP induced ROS production.
- The data suggest that glycolysis drives ROS production and neuronal excitability

References:

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3. Fisher, R.S., van Emde Boas, W., Blume, W. Elger, C., Genton, P., Lee, Pc, and Engel, J., Jr. (2005) Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureu for Epilepsy (IBE). Epilepsia 46, 470-472.

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