Abstract

Metabolic control of epilepsy is recognized in part due to the efficacy of ketogenic diets, which provide alternative 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 (measured of glycolytic rates 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 glycogen substrate limitation), nicotinamide riboside (NR) – an NAD+ precursor, exokaseutax (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, PKD 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

NEURONAL EXCITABILITY ➔ GLYCOLYSIS ➔ REACTIVE OXYGEN SPECIES

Background

Previous Work Indicating Oxidative Stress in Epileptogenesis

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

Production and Detoxification of Reactive Oxygen Species

Potassium Channel Blockers to Induce Neuronal Excitability

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

Results

Pharmacological Inhibition of 4-AP-induced Glycolytic Rates

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

Methods

Rat Cortical Culture

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 glycolic 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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