Seizure-induced differential protein expression of pfkfb3 and pdha1

Seizure-induced Differential Protein Expression of Pfkfb3 and Pdha1in Mice Possessing or Lacking GYS1

The brain has high energy requirements in comparison to the rest of the body and is tightly regulated to ensure proper neuronal activity. The primary source of energy for the brain is glucose [1]. Glucose can be stored in the brain in the form of glycogen. Glycogen is a multibranched polysaccharide that can be rapidly metabolized to maintain energy homeostasis. The protein glycogen synthase 1 (GYS1) catalyzes the addition of glucose to growing glycogen molecule in skeletal muscle while GYS2 does the same in the liver [2, 3]. Mutations of the liver and muscle isoform of glycogen synthase have been linked to hypoglycemia, muscle weakness, and cardiomyopathy due to glycogen storage deficiencies [4, 5]. Although glycogen is the most abundant energy reserve in the brain, it is almost exclusively found in astrocytes [6]. It is currently hypothesized that glycogen stored in astrocytes preserve neuronal function during times of neuronal stress (e. g. hypoglycemia and seizures) by providing neurons energy in the form of lactate using a neuron-lactate shuttle [7].

Neurons can also obtain glucose through glucose transporters (GLUTs). This glucose will undergo glycolysis by first being phosphorylated by hexokinase to glucose-6-phosphate and converted to Fructose 6-phosphate (Fructose-6P) [8]. Once converted to fructose-6P, 6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 3 (Pfkfb3) can either catalyze the synthesis or degradation of fructose-2, 6-bisP (F2, 6BP) [9]. This conversion is the primary rate-limiting step of glycolysis and is irreversible. Once converted, F2, 6BP activates 6-phosphofructokinase-1 (PFK-1), which will stimulate glycolysis. Pfkfb3 also

promotes cell cycle progression and suppresses apoptosis by activating Cdk-1 and decreasing p27 levels [10]. Taken together, Pfkfb3 is a key regulator of glycolysis plays an essential role in neuronal energy metabolism, cell proliferation, and cell death.

An end product of glycolysis is pyruvate, which either can be sent to the mitochondria or can be converted to lactate. Pyruvate enters the mitochondria through use of the pyruvate dehydrogenase complex (PDC) [11]. The PDC converts pyruvate into acetyl-CoA, which is then metabolized in the tricarboxylic acid (TCA) cycle and oxidative phosphorylation. Pyruvate dehydrogenase E1 alpha 1 subunit (Pdha1) is a subunit of the PDC and is responsible for the enzymatic activity of the complex [12]. PDC deficiencies due to Pdha1 mutations cause encephalopathy, Leigh syndrome, and relapsing ataxia [13, 14]. Notably, failure of the PDC leads to neuronal injury and impairment [15]. These studies suggest the PDC, namely the Pdha1 subunit, play an essential role in preventing seizures and promoting neuronal survival. However, emerging data suggests that the end product of glycolysis in the neuron is lactate instead of pyruvate [16]. Instead of pyruvate being shuttled into the mitochondria, a putative mitochondrial complex allows lactate entry and for subsequent oxidation in the mitochondria [17]. This is perhaps unsurprising because neurons efficiently use lactate and even prefer it over glucose when both are present [18]. Overall, pyruvate and lactate are key substrates that provide the neuron with energy; however, their role and the neurons preference for either substrate for energy when under neuronal stress is currently unknown.

In this study, we seek to elucidate the function of Pdha1 and Pfkfb3 in the brain of glycogen deficient mice when introduced to pilocarpine-induced cell stress under hypoglycemic conditions by observing expression and localization in the hippocampus. We hypothesize that Pfkfb3 will be initially upregulated to promote anaerobic glycolysis due to Pfkfb3 expression being induced by hypoxia [19]. The neurons will be in a hypoxic environment due to the increase in aerobic metabolism (TCA cycle) necessary to maintain energy supplies during seizures [20]. This increase in anaerobic glycolysis will lead to an increase in lactate. Lactate production has been found to regulate Pfkfb3 activity by dissociating the enzyme active tetramers into less active dimers, inhibiting glycolysis [20]. This inhibition then terminates seizures due to a shortage of energy supplies. We also anticipate that Pdha1 will be downregulated due to less pyruvate being produced by glycolysis. This downregulation would also support previous studies in which it was concluded that glycolysis produces lactate instead of pyruvate. Pilocarpine application has also been found to lead to a rise in lactate, not pyruvate [21]. Our work will expand on previous work on how the neuron survives stress caused by seizures and epilepsy by examining the regulators of glycolysis and the TCA cycle. If Pdha1 is downregulated, then it likely indicates it is not a potential target for drug treatment, and the mitochondrial lactate shuttle system should instead be further pursued.

References:

1. Bélanger, M., I. Allaman, and P. J. Magistretti, *Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation.* Cell metabolism, 2011. 14(6): p. 724-738.

2. Hunter, R. W., et al., *Expression and purification of functional human glycogen synthase-1: glycogenin-1 complex in insect cells.* Protein expression and purification, 2015. 108: p. 23-29.

3. Kaslow, H. R. and D. D. Lesikar, *Isozymes of glycogen synthase.* FEBS letters, 1984. 172(2): p. 294-298.

4. Orho, M., et al., *Mutations in the liver glycogen synthase gene in children with hypoglycemia due to glycogen storage disease type 0.* The Journal of clinical investigation, 1998. 102(3): p. 507-515.

5. Kollberg, G., et al., *Cardiomyopathy and exercise intolerance in muscle glycogen storage disease 0.* New England Journal of Medicine, 2007. 357(15): p. 1507-1514.

6. Magistretti, P., O. Sorg, and J. Martin, *Regulation of glycogen metabolism in astrocytes: physiological, pharmacological, and pathological aspects.* Astrocytes: pharmacology and function, 1993: p. 243-265.

7. Brown, A. M. and B. R. Ransom, *Astrocyte glycogen and brain energy metabolism.* Glia, 2007. 55(12): p. 1263-1271.

8. Clarke, D. D., *Circulation and energy metabolism of the brain.* Basic neurochemistry: Molecular, cellular, and medical aspects, 1999.

9. Watanabe, F., A. Sakai, and E. Furuya, *Novel Isoforms of Rat Brain Fructose 6-Phosphate 2-Kinase/Fructose 2, 6-Bisphosphatase Are Generated by Tissue-Specific Alternative Splicing.* Journal of neurochemistry, 1997. 69(1): p. 1-9.

10. Yalcin, A., et al., *6-Phosphofructo-2-kinase (PFKFB3) promotes cell cycle progression and suppresses apoptosis via Cdk1-mediated phosphorylation of p27.* Cell death & disease, 2014. 5(7): p. e1337.

11. Addink, A. D., et al., *Enzyme Localization in Beef-Heart Mitochondria: A Biochemical and Electron-Microscopic Study.* European journal of biochemistry, 1972. 29(1): p. 47-59.

12. Chun, K., et al., *Mutations in the X-linked E1 alpha subunit of pyruvate dehydrogenase: exon skipping, insertion of duplicate sequence, and missense mutations leading to the deficiency of the pyruvate dehydrogenase complex.* American journal of human genetics, 1995. 56(3): p. 558.

13. Barnerias, C., et al., *Pyruvate dehydrogenase complex deficiency: four neurological phenotypes with differing pathogenesis.* Developmental Medicine & Child Neurology, 2010. 52(2): p. e1-e9.

14. Wexler, I., et al., *Outcome of pyruvate dehydrogenase deficiency treated with ketogenic diets Studies in patients with identical mutations.* Neurology, 1997. 49(6): p. 1655-1661.

15. Martin, E., R. E. Rosenthal, and G. Fiskum, *Pyruvate dehydrogenase complex: metabolic link to ischemic brain injury and target of oxidative stress.* Journal of neuroscience research, 2005. 79(1-2): p. 240-247.

16. Schurr, A. and R. Payne, *Lactate, not pyruvate, is neuronal aerobic glycolysis end product: an in vitro electrophysiological study.* Neuroscience, 2007. 147(3): p. 613-619.

17. Hashimoto, T., et al., *Evidence for the mitochondrial lactate oxidation complex in rat neurons: demonstration of an essential component of brain lactate shuttles.* PloS one, 2008. 3(8): p. e2915.

18. Bouzier-Sore, A. K., et al., *Competition between glucose and lactate as oxidative energy substrates in both neurons and astrocytes: a comparative NMR study.* European Journal of Neuroscience, 2006. 24(6): p. 1687-1694.

19. Minchenko, A., et al., *Hypoxia-inducible Factor-1-mediated Expression of the 6-Phosphofructo-2-kinase/fructose-2, 6-bisphosphatase-3 (PFKFB3) Gene ITS POSSIBLE ROLE IN THE WARBURG EFFECT.* Journal of Biological Chemistry, 2002. 277(8): p. 6183-6187.

20. Yang, H., et al., *Glycolysis in energy metabolism during seizures.* Neural regeneration research, 2013. 8(14): p. 1316.

21. Slais, K., et al., *Brain metabolism and diffusion in the rat cerebral cortex during pilocarpine-induced status epilepticus.* Experimental neurology, 2008. 209(1): p. 145-154.