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Mitochondrial detachment of hexokinase 1 in mood and psychotic disorders: Implications for brain energy metabolism and neurotrophic signaling

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ABSTRACT

The pathophysiology of mood and psychotic disorders, including unipolar depression (UPD), bipolar disorder (BPD) and schizophrenia (SCHZ), is largely unknown. Numerous studies, from molecular to neuroimaging, indicate that some individuals with these disorders have impaired brain energy metabolism evidenced by abnormal glucose metabolism and mitochondrial dysfunction. However, underlying mechanisms are unclear. A critical feature of brain energy metabolism is attachment to the outer mitochondrial membrane (OMM) of hexokinase 1 (HK1), an initial and rate-limiting enzyme of glycolysis. HK1 attachment to the OMM greatly enhances HK1 enzyme activity and couples cytosolic glycolysis to mitochondrial oxidative phosphorylation, through which the cell produces most of its adenosine triphosphate (ATP). HK1 mitochondrial attachment is also important to the survival of neurons and other cells through prevention of apoptosis and oxidative damage. Here we show, for the first time, a decrease in HK1 attachment to the OMM in postmortem parietal cortex brain tissue of individuals with UPD, BPD and SCHZ compared to tissue from controls without psychiatric illness. Furthermore, we show that HK1 mitochondrial detachment is associated with increased activity of the polyol pathway, an alternative, anaerobic pathway of glucose metabolism. These findings were observed in samples from both medicated and medication-free individuals. We propose that HK1 mitochondrial detachment could be linked to these disorders through impaired energy metabolism, increased vulnerability to oxidative stress, and impaired brain growth and development.

1. Introduction

The pathophysiology of both mood and psychotic disorders, including unipolar major depression (UPD), bipolar disorder (BPD) and schizophrenia (SCHZ), is largely unknown. A variety of studies, from molecular to neuroimaging, now implicate impaired brain energy metabolism, evidenced by abnormal brain glucose metabolism and mitochondrial dysfunction in the pathophysiology of these disorders (Cataldo et al., 2010; Dager et al., 2004; Holmes et al., 2006; Kato and Kato, 2000; Konradi et al., 2004; Kung and Roberts, 1999; Prabakaran et al., 2004; Quiroz et al., 2008; Regenold et al., 2004, 2009; Shao et al., 2008; Sun et al., 2006; Valvassori et al., 2010; for recent reviews see Kato et al., 2010 and Clay et al., 2011) Although this evidence suggests a relationship to pathophysiology, underlying mechanisms have not been worked out. Consequently, clear rationales for specific treatments aimed at improving brain energy metabolism in these disorders are lacking.

This study investigated a potential mechanism underlying impaired brain energy metabolism. We hypothesized that detachment of hexokinase 1 (HK1) from the outer mitochondrial membrane (OMM) contributes to impaired brain energy metabolism in mood and psychotic disorders. Hexokinase (HK) is an initial and rate-limiting enzyme of glycolysis, and HK1 is the major isozyme of HK found in brain. HK1 is unique among the four known isozymes in being the only one that is predominantly bound to mitochondria. HK1 is normally 75–90% bound in neurons and astrocytes depending on the animal species and tissue characteristics (Crane and Sols, 1953; Hutny and Wilson, 2000; Lynch et al., 1991; Oudard et al., 1995; Wilson, 2003). HK1 attaches by reversibly binding to the OMM protein, porin, also known as the voltage dependent anion channel (VDAC) (Fig. 1). Two isoforms of VDAC have been identified in human brain, VDAC1 and VDAC2. HK1 has been shown to bind VDAC1 via its hydrophobic, N-terminal

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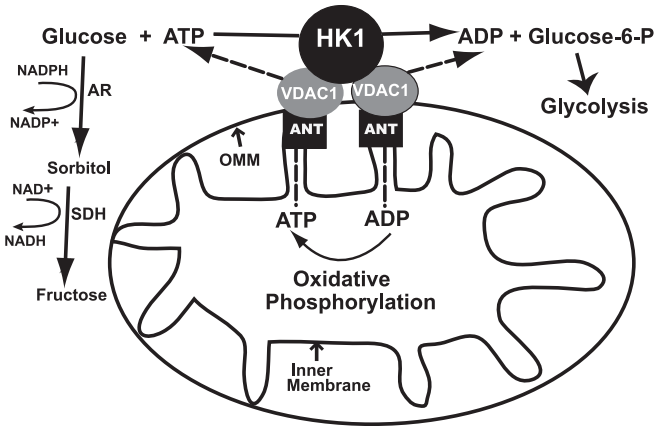

Fig. 1. The coupling of mitochondrial oxidative phosphorylation to cytosolic glycolysis by attachment of hexokinase 1 (HK1) to the outer mitochondrial membrane (OMM) through binding to the voltage dependent anion channel (VDAC1) which in turn interacts with a portion of the adenine nucleotide translocases (ANT) within the inner mitochondrial membrane. The polyol pathway of glucose metabolism is shown to the left. Glucose is reduced to sorbitol via aldose reductase (AR) and hydrogen from nicotinamide adenine dinucleotide phosphate (NADPH). Sorbitol is then oxidized via sorbitol dehydrogenase (SDH) forming nicotinamide adenine dinucleotide (NADH) and fructose.

sequence. VDAC1 in turn interacts with a portion of the adenine nucleotide translocases (ANT) within the inner mitochondrial membrane. HK1 attachment to the OMM greatly increases HK1 enzyme activity and couples cytosolic glycolysis to mitochondrial oxidative phosphorylation, which produces 95% of the adenosine triphosphate (ATP) needed to meet the extraordinary energy demands of the brain (Erecinska and Silver, 1989). When HK1 binds VDAC1, the channel opens, allowing mitochondrial ATP to migrate to the mitochondrial/cytosolic interface where it donates a high energy phosphate to glucose in the HK1-catalyzed, initial, rate-limiting reaction of glycolysis. ADP is then recycled back into the mitochondrion through VDAC, and HK1 is available to accept another high energy phosphate. Mitochondrially bound HK1 therefore has privileged access to mitochondrial ATP, enhancing its activity and functionally coupling glucose phosphorylation to mitochondrial ATP production (de Cerqueira Cesar and Wilson, 1995). This coordination between cytosolic glycolysis and mitochondrial oxidative phosphorylation enables a rate of glucose metabolism that matches cellular energy demands and avoids the excessive production of lactate (Wilson, 2003). HK1 detachment disrupts this cycle, greatly decreasing oxidative phosphorylation and therefore ATP, resulting in compromised cell energy metabolism (BeltrandelRio and Wilson, 1992; Saraiva et al., 2010).

HK mitochondrial attachment also plays critical roles in cell survival and protection against oxidative damage. For this reason mitochondrially attached hexokinases have been called “guardians of the mitochondria” (Robey and Hay, 2005). HK mitochondrial attachment is a downstream effector of the growth factor-mediated phosphatidyl inositol 3-kinase (PI_3)/Akt cell survival intracellular signaling pathway (Gottlob et al., 2001). Attachment has been shown to prevent mitochondrially-induced apoptosis (Majewski et al., 2004) and to prevent oxidative damage by reducing mitochondrial reactive oxygen species (ROS) (da-Silva et al., 2004).

The rationale for our hypothesis included evidence from genetic studies as well as from our prior studies of brain glucose metabolism in these disorders. Microarray and large genetic linkage studies have found abnormalities of expression of, and linkage to, the HK1 gene (Olsen et al., 2008; Stone et al., 2004) and more frequently to the chromosomal regions containing the HK1 gene (10q22) (Fallin et al., 2004; Faraone et al., 2006; Marcheco-Teruel et al., 2006; Venken et al., 2008) and the VDAC1 gene (5q31) (Herzberg et al., 2006; Hong et al., 2004; Sklar et al., 2004) in BPD and SCHZ. In prior studies of cerebrospinal fluid (CSF) and postmortem parietal cortex tissue of individuals with these disorders, we found evidence of increased activity of non-mitochondrial, anaerobic pathways of glucose metabolism—the polyol pathway and glycolysis ending in lactate production (Regenold et al., 2008, 2000, 2004, 2009). Impaired HK1 activity that occurs with HK1 mitochondrial detachment shunts glucose to competing pathways of glucose metabolism, including the polyol pathway (Fig. 1) (Jeffery and Jornvall, 1983), which has been reported to be upregulated in a cellular model of mitochondrial disorder (Danielson et al., 2005). Increased flux of glucose through the polyol pathway causes intracellular accumulation of the metabolite, sorbitol, which causes direct tissue toxicity and swelling through an osmotic mechanism (Gabbay et al., 1966; Tomlinson and Gardiner, 2008). In order to replicate our findings, for this study we used tissue from the same parietal cortex region, a region implicated by neuroimaging studies in all three disorders (Biver et al., 1994; Cleghorn et al., 1989; El-Sayed et al., 2010; Kishimoto et al., 1987; Mah et al., 2007; Maruff et al., 2005; Ojeda et al., 2002; Ongur et al., 2010; Soriano-Mas et al., 2011). We further hypothesized that increased concentrations of polyol pathway metabolites would correlate inversely with the degree of HK1 mitochondrial attachment. As a control, we analyzed tissue from the primary motor cortex as differences between individuals with mood and psychotic disorders and controls have not been consistently reported in this region.

2. Materials and methods

2.1. Postmortem brain samples

This study was reviewed and approved by the University of Maryland, Baltimore Institutional Review Board. We used postmortem fresh-frozen, parietal somatosensory association (BA7) and primary motor (BA4) cortex tissue blocks from the Stanley Neuropathological Consortium (Rockville, MD, USA). We studied brains from individuals with the following disorders: SCHZ ($N = 15$), BPD ($N = 15$), nonpsychotic UPD ($N = 15$, parietal and $N = 14$, motor), and unaffected controls without psychiatric illness ($N = 15$). Samples were stored at −70 to −80 °C until use. Experiments were done blind to demographic, clinical and brain-related information. Samples were matched for age, race, gender, brain pH, postmortem interval (PMI), and side of brain. Brain pH was obtained by investigators at the Stanley Medical Research Institute (Chevy Chase, MD, USA). The mean (±standard deviation) values for age, pH, and PMI were 45.4 ± 11.2 years, 6.20 ± 0.23, and 29.4 ± 13.4 h, respectively. Sixty percent of individuals were male, and racial composition was: Caucasian ($N = 56$), African American ($N = 2$), and Asian ($N = 2$). Demographic and clinical characteristics of individuals and methods of brain tissue processing have been published in detail elsewhere by Torrey et al. (2000). Thirty-seven of the 45 individuals with psychiatric illness (44 for motor cortex) had been taking CNS medications at the time of death, and eight had been free of CNS medications for at least several months prior to death.

2.2. Tissue preparation

Tissue for western blots was thawed on ice, gray matter was dissected from white matter and blood vessels, and then homogenized in ice-cold isolation medium containing Tris–HCl 10 mM,