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Research Report

Brain glucose transporter (Glut3) haploinsufficiency does not impair mouse brain glucose uptake

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ABSTRACT

Mouse brain expresses three principal glucose transporters. Glut1 is an endothelial marker and is the principal glucose transporter of the blood-brain barrier. Glut3 and Glut6 are expressed in glial cells and neural cells. A mouse line with a null allele for Glut3 has been developed. The $Glut3^{-/-}$ genotype is intrauterine lethal by 7 days post-coitis, but the heterozygous ($Glut3^{+/-}$) littermate survives, exhibiting rapid post-natal weight gain, but no seizures or other behavioral aberrations. At 12 weeks of age, brain uptake of tail vein-injected ^{3}H-2-deoxy glucose in $Glut3^{+/-}$ mice was not different from $Glut3^{+/+}$ littermates, despite 50% less Glut3 protein expression in the brain. The brain uptake of injected ^{18}F-2-fluoro-2-deoxy glucose was similarly not different from $Glut3^{+/-}$ littermates in the total amount, time course, or brain imaging in the $Glut3^{+/-}$ mice. Glut1 and Glut6 protein expressions evaluated by immunoblots were not affected by the diminished Glut3 expression in the $Glut3^{+/-}$ mice. We conclude that a 50% decrease in Glut3 is not limiting for the uptake of glucose into the mouse brain, since Glut3 haploinsufficiency does not impair brain glucose uptake or utilization.

1. Introduction

Facilitative hexose transporters are part of a family of solute carriers termed SLC2 with 14 different isoforms, most of which are designated Glut's and transport glucose (Joost et al., 2002). The first of these proteins, Glut1, was identified and cloned in 1985. The third member, Glut3, was also named the "brain glucose transporter" because it was expressed at high levels in nerves and neural tissue. Glut3 is also found at lower expression levels in many other tissues, including muscle and fat (Bell et al., 1993). Like many of the proteins of this family, Glut3 is expressed primarily on the cell surface and is not acutely regulated by insulin in the "insulin-sensitive" tissues, muscle and fat (Scheepers et al., 2004). Since it is likely that Glut3 provides at least a portion of the basal glucose uptake in muscle and fat (Simpson et al., 2008; Stuart et al., 2001), we developed a Glut3 knockout mouse line to investigate the impact on insulin responsiveness of diminished basal

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glucose uptake in muscle and fat. In agreement with other reports (Ganguly et al., 2007; Schmidt et al., 2009), the Glut3$^{-/-}$ conceptus in our line does not survive beyond E7 days post-coitis (dpc), but the heterozygous littermates survive and are fertile (Stuart et al., 2007). As part of the characterization of the phenotype of the Glut3$^{+/-}$ mice, we documented that Glut3 protein expression in brain was 50% of that of the Glut3$^{+/+}$ littermates. To our surprise, there was no change in behavior or seizure activity as seen in Glut1$^{+/-}$ mice, even with prolonged fasting in the heterozygous mice. This report details our investigation of the brain glucose uptake in Glut3$^{+/-}$ mice.

2. Results

2.1. *Expression of Glut1, Glut3, and Glut6 in Glut3$^{+/-}$ mouse brain*

Fig. 1 displays sample immunoblots of mouse brain homogenate probed with antibodies directed against mouse Glut3, human Glut1, and human Glut6. The Glut1 antibody is fully active against mouse Glut1 since the 12 amino acid peptide used to generate the antibody is identical to the carboxy terminus of both human and mouse. The Glut6 antibody was generated with a 16 amino acid peptide that has 56% identity with the mouse carboxy terminal sequence. This antibody was specifically reactive with the mouse Glut6. The Glut3$^{+/-}$ mouse brain contained 52% of the level of signal seen in the Glut3$^{+/+}$ littermates. Neither Glut1 nor Glut6 immunoblots indicated any increase in expression in the Glut3$^{+/-}$ as compared to homogenate from Glut3$^{+/+}$ mouse brains. Glut1 expression in Glut3$^{+/-}$ mice averaged 16% less and Glut6 was 15% more than Glut3$^{+/+}$ littermates, but neither of these differences were statistically significant. Thus, no evidence was found for compensatory post-translational increase in Glut3 expression or compensation through increases in either of the other two principal glucose transporters expressed in the brain.

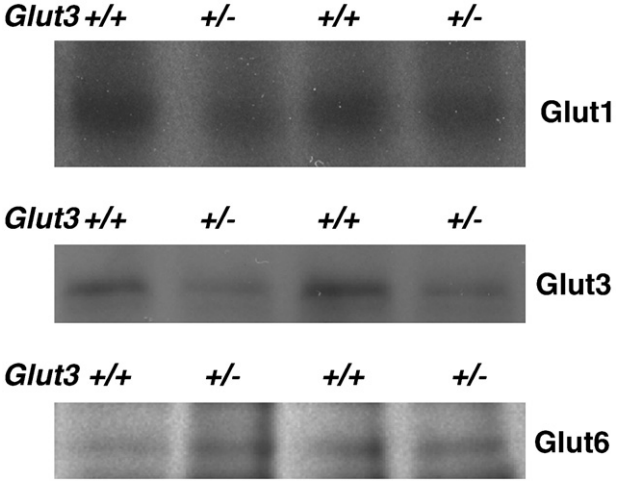

Fig. 1 – Expression of Glut1, Glut3, and Glut6 in brain homogenate from Glut3$^{+/-}$ mice. Shown here are representative immunoblots of brain homogenates subjected to PAGE and probing of resulting membranes with antibodies against mGlut3, hGlut1, and hGLlut6. Digital image analysis quantified the mean expression of Glut3 at 52% of the Glut3$^{+/+}$ littermates. The expressions of Glut1 and Glut6 in Glut3$^{+/-}$ brain were quantified at 100% and 100%, respectively.

2.2. *Prolonged fasting of Glut3$^{+/-}$ mice results in decreased blood glucose concentrations but no observable seizure activity*

Since heterozygous defects in the *Glut1* gene are associated with a 50% decrease in Glut1 and neonatal seizures in humans (De Vivo et al., 1991) and mice (Wang et al., 2006), we speculated that a similar decrease in Glut3 expression in brain might parallel the effects of a 50% decrease in the glucose transporter that constitutes the blood-brain barrier (Glut1). Male and female mice with Glut3$^{+/-}$ or Glut3$^{+/+}$ genotypes were subjected to restricted food access in order to decrease the blood glucose concentration and cause a decrease in the cerebrospinal fluid concentration of glucose. Fig. 2 displays the changes that occurred in blood glucose in animals subjected to food deprivation up to 48 h. Ambient temperature in the animal care facility room was maintained at 29 °C for 6 weeks in order to minimize the decrease in physical activity that occurs with limited food. By 24 h, blood glucose declined to 60–80 mg/dl, about half of that of the ad lib fed mice at the beginning of this study. The activity of the mice decreased with food deprivation, but there was no evidence of seizure activity or behavior arrest and the behavior of the mice was otherwise indistinguishable between Glut3$^{+/-}$ and Glut3$^{+/+}$ genotypes.

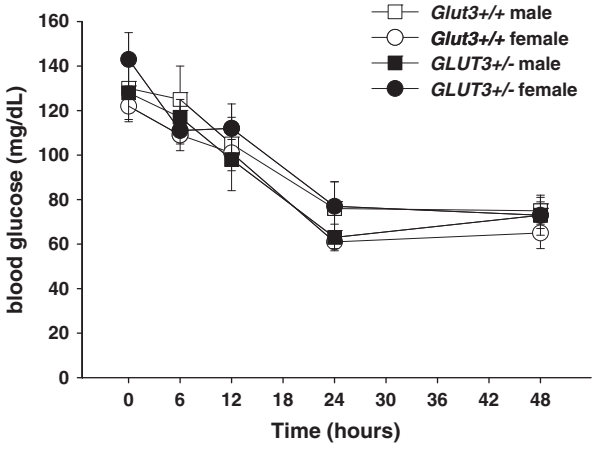

Fig. 2 – Tolerance to prolonged fasting in mice with Glut3$^{+/-}$ genotype. Mice at 10–12 weeks of age were divided into four groups of four animals, male Glut3$^{+/-}$, female Glut3$^{+/-}$, male Glut3$^{+/+}$, and female Glut3$^{+/+}$. After ad lib feeding overnight, food was removed and glucose was determined in blood from a cheek puncture at times 0, 6, 12, 24, and 48 h. Each mouse had single blood glucose determinations at five different times separated by 1 week before the next sequentially increased fasting time period. Ambient temperature was maintained at 29 °C to minimize torpor that can occur with food restriction. At no time was there evidence of seizure activity or other change in behavior among the mice during the 48 h of frequent observation. No significant difference in glucose concentrations was found comparing the groups at any of the durations of fasting.