Short-echo-time ^{1}H MRS of the mouse lacking brain-specific glutamate-dehydrogenase

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Introduction

The abundance of genetic models for studying normal function and malfunction of the brain implicates the study of mouse brain by magnetic resonance, which is a method uniquely suited to translate these measurements to humans. Glutamate (Glu) is the major excitatory neurotransmitter and its maintenance is critical for normal function. Glutamate-dehydrogenase (GDH), along with aspartate aminotransferase, is an enzyme implicated in the homeostasis of the brain Glu concentration. Recent studies have suggested an involvement of anaplerosis in the maintenance of cerebral Glu concentrations (1).

The measurement of proton short-echo-time localized spectra is reportedly more challenging in mice than in rats, in particular due to increased demands on shimming (2,3). Therefore, the aim of the present study was to use high-resolution ^{1}H MRS to measure changes in the Glu and glutamine (Gln) concentrations, and the neurochemical profile in mice, whose brain GDH isoform had been knocked out.

Methods

In order to evaluate the role of GDH in brain metabolism, brain-specific GDH knockout mice were created. GDH was deleted by crossing Exon-7 GDH floxed mice with Nestin-Cre mice. The mice were group-housed on a 12h/12h day/night cycle at 20 ± 2 °C and at a relative air humidity of 50 ± 10 %. They were fed standard mouse pellets. Anesthesia was maintained at 1.3 ± 0.2 % of isoflurane in oxygen, body temperature was kept at 36.5 ± 0.2 °C. Spectra were acquired on an actively shielded 9.4 T/31 cm INOVA imaging spectrometer (Varian/Magnex Scientific) using the SPECIAL spectroscopy sequence (4). This technique enables to measure short-echo-time spectra with full signal intensity available in the excited volume. A 14 mm diameter two-loop quadrature coil was used both for RF excitation and signal reception. Field homogeneity was adjusted using the FASTMAP protocol (5). A VOI having the nominal size of 22 to 30 microliters (typically 3 mm×2.5mm ×3 mm) was selected in striatum, TR was 4 s and TE was 2.7 ms. Signal from the outer volume was suppressed by four blocks of slice selective pulses, water signal was suppressed by the VAPOR sequence (6). 160 scans collected in 10 minutes provided spectra with excellent SNR. The measured spectra were not eddy-current corrected. Absolute concentration of metabolites were obtained by LCModel using unsuppressed water signal as a reference (7). The Cramer-Rao lower bounds for concentrations of the most abundant metabolites were 1-2 %.

Results

In most mice, shimming the volume of interest in striatum using FASTMAP was within 50 % of the provided maximum shim strength and the water resonance FWHM was 13-14 Hz, resulting in ^{1}H MR spectra with excellent resolution (Fig. 1). The present study showed that with increased shim capabilities, previously reported shim limitations could be overcome, which resulted in high spectral quality allowing the resolved detection of PCr and Cr (Fig. 1). Concentrations of the metabolites (neurochemical profile, Fig. 2) were in good agreement with previous results (2). In the knockout mice the overall neurochemical profile was similar to the wild-type. Specifically, aspartate (Asp) and Glu changes were within experimental error. However, Gln was elevated ($P < 0.03$).

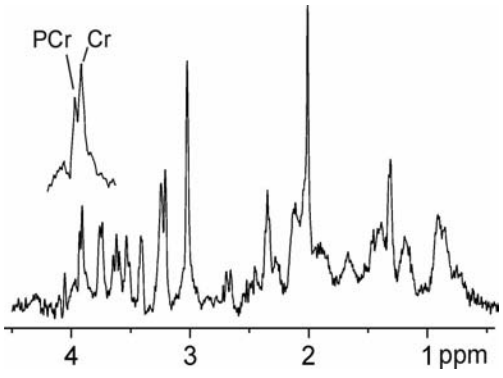

Fig. 1. A SPECIAL proton spectrum of the mouse striatum, VOI = 30 µl, 160 scans

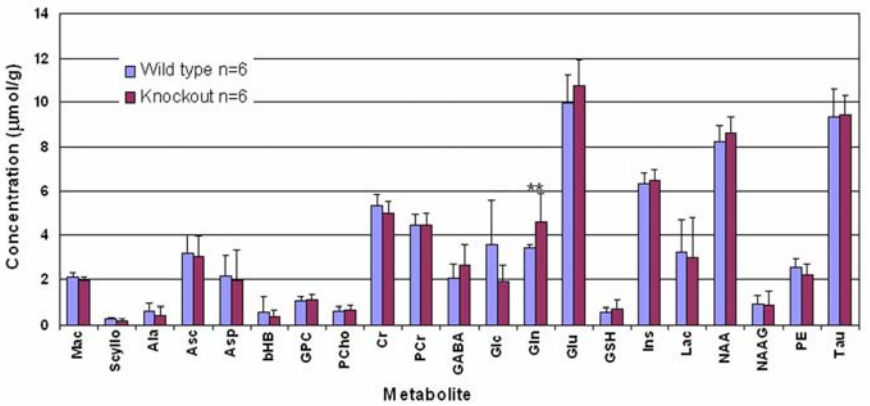

Fig. 2. Neurochemical profile (mean ± SD) in the mouse striatum

Discussion

Compared to the wild-type mice, the GDH transgenic mice show a small increase in the Gln concentration. The observation of overall small changes in the neurochemical profile, specifically the amino acids linked to Glu metabolism (Glu, Gln and aspartate) suggest a modest effect of GDH knockout on amino acid homeostasis. Since transamination results in no net change of amino acid concentration, this implies that other processes, such as transport of aminoacids across the blood-brain barrier, or other GDH isoenzymes must contribute to Glu homeostasis in the resting brain. It remains to be determined whether the knockout of GDH would exacerbate, e.g., hypoglycemic injury.

Acknowledgments

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