Analysis of NAA in gene NAT8l knock out mice using proton Magnetic Resonance Spectroscopy

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INTRODUCTION

N-acetylaspartate (NAA) is the second most concentrated chemical in the human brain, and yet more than 50 years after it's discovery, the physiological functions served by NAA are still a matter of strong debate and intensive research. NAA levels in the brain can be studied non-invasively by magnetic resonance spectroscopy (MRS), and numerous MRS studies during the last two decades have shown that NAA is decreased in specific neuronal systems in a number of neurological and psychiatric disorders and in substance abuse conditions. We have recently identified NAT8l as the most likely gene that codes for the biosynthetic enzyme of NAA and have generated heterozygous *Nat8l* knockout mice for our studies, but for reasons that are presently unclear these mice do not breed, even in the case of heterozygotes. This is not unexpected based on the widespread distribution of NAA in neurons, including in many hypothalamic nuclei. These results further indicate that NAA is not just a surrogate marker, but rather that it plays critical roles in CNS function, the details of which remain to be investigated. Here we characterize NAA concentrations in localized brain regions of the NAT8l knockout mouse by using proton magnetic resonance spectroscopy (1H-MRS).

METHODS

Two groups of 4 mice (male, 14-15 months old, control and NAT8l knockout) were used in this experiment. In vivo 1H-MRS was performed on a Bruker BioSpec system (Bruker NMR, Inc., Billerica, MA) consisting of a 7-Tesla (T), 20-cm horizontal bore, superconducting magnet (Magnex Scientific, Abingdon UK) with a Biospec 70/20 console and Paravision software. An Autopac mouse positioning and physiological monitoring system, 86mm quadrature transmit coil and a 4-channel phase array mouse head coil were used. Mice were anesthetized with isoflurane during the preparation period and during the scan using a 'flow through' nose cone (1.5-2.0%). T2 weighted high resolution images (RARE, 0.3x0.3x0.25mm, TE=15,45ms, matrix=128x128x100) were acquired at the coronal, sagital, and axial orientations with the whole brain coverage. Single voxel 1H-MRS data was acquired using Point RESolved Spectroscopy (PRESS, TR/TE = 2500/20ms, averages = 256, voxel size = 3-5mm^3), with and without water suppression, localized in the cortex, hypothalamus, and cerebellum. Spectra were processed and displayed using the XSOSNMR software (X. Mao & D. Shungu, Laboratory for Advanced MRS Research, Cornell University) and in LCModel (S.W. Provencher) for quantitation, with eddy current correction and water-scaling for NAA, Creatine (Cr), Choline (Cho) and Glutamate+Glutamine (Glx). Concentrations between groups were statistically analyzed in MATLAB (The Math Works, Natick, MA) and GraphPad (GraphPad, La Jolla, CA) for significance.

RESULTS

Major results of the study are summarized in the Table. NAA in control versus NAT8l knockout mice decreased 38% in the cortex (Figure), 29% in the hypothalamus and 8% in the cerebellum. Unpaired t-test results in both the cortex and hypothalamus show significant differences in mean NAA concentrations (p=0.0142), while the cerebellum showed least significance for NAA. Cr increased significantly in both the hypothalamus (p=0.0747) and cerebellum (p=0.0940), with a 27% increase in the later. Both Cho and Glx showed no significant changes in any brain region (p values > 0.5).

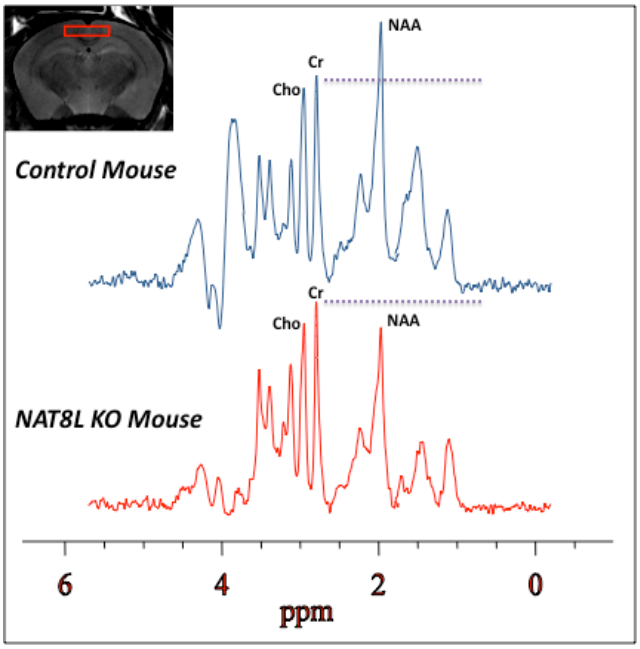

Figure: Spectra of control and NAT8L KO mice from the cortex. Dashed lines represent Cr peak level.

Average % Difference, knockout - control (p-value of unpaired t-test)

	[NAA]	[Cr]	[Cho]	[Glx]
Cortex	-37.99% (0.0142)	16.92% (0.2130)	9.94% (0.5492)	12.35% (0.4997)
Hypothalamus	-28.53% (0.0004)	10.82% (0.0747)	1.79% (0.7995)	2.71% (0.7027)
Cerebellum	-7.88% (0.1111)	26.50% (0.0940)	-4.24% (0.6500)	-2.01% (0.7661)

Table: Average % difference between NAT8L knockout and control mice. P-values listed from unpaired t-test.

DISCUSSION

NAT8l knockout heterozygote mice have significantly decreased NAA levels in the brain. The lack of significant decrease in the cerebellum suggests that regulation of expression of NAT8l gene might differ in different areas of the brain. Increases in Cr levels require future investigation. Further studies using these animals will be important for understanding the functional roles of NAA in the brain and its involvement in neurological and mental disorders.

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