Gy mice lacking spermine show reduced taurine in brain hippocampus detected by 1H MRS

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Introduction:

The polyamines putrescine, spermidine, and spermine are essential for cell growth, differentiation and cell death and have important roles in different tissues. The precise roles of polyamines are still largely unknown. Spermine synthase **(SpmS)** is 1 of 5 enzymes involved in the synthesis of polyamines from ornithine and methionine. A greatly reduced SpmS activity in humans is the cause of Snyder-Robinson syndrome, an X-linked mental retardation condition. Animal models for SpmS studies include the Gy mouse and transgenic mouse (CAGSpmS) that overexpresses SpmS from a ubiquitous promoter. Gy males have a deletion of the SpmS gene; therefore, they lack SpmS activity and have no spermine and increased spermidine. They have neurological deficiencies including a typical circling behavior. Gy males also have decreased life span, stunted growth and are sterile. CAGSpmS mice have a normal life span and are fertile. Four lines of CAGSpmS have been studied. Lines #8, #21 and #5 show widespread overexpression of SpmS in many organs including brain. Breeding of line #8 or #21 with the Gy mice reverses the phenotypes describe above. Breeding with line #18, which gives a lower expression of spermine synthase in brain, did not reverse the phenotype.

In order to further understand the function of spermine, the presence and distribution of SpmS in different mouse tissues has been identified by immunohistochemistry methods. This light microscope study revealed that SpmS is expressed in the mouse brain hippocampus region. The purpose of this study is two-fold: 1) to determine the sensitivity of MRS in the mouse hippocampus, and 2) to determine the metabolic changes after the SpmS gene is altered in the mouse brain.

Methods:

Animal: Three Gy mice (5 months old) and three age-matched control littermates (5 months old) were recruited into this study.

^{1}H NMR Spectroscopy: All experiments were performed on a 7.0 T/21cm interfaced to a Bruker Topspin console. Volume coil and surface coil were used for a transmitter and a receiver, respectively. Breathing mice were anesthetized by the flow of a gas mixture (flow rate 0.8 liter/min) containing 1.5-2% isoflurane. T2-weighted image (RARE sequence, rare factor= 8, TR= 4200ms, TE= 36ms, average= 5, matrix size = 256×196, filed of view (FOV)= 2.5cm × 2.5cm, slice thickness= 0.5mm, slice number= 20) was used for the positioning of the single volume of interest within the hippocampus ($1.7\times1.7\times1.7mm^3$). Field inhomogeneity was optimized using the Fastmap shimming technique. Localization was achieved by a short echo PRESS sequence (TE= 8ms, TR= 4000ms, number of points= 1024, spectral width = 5000 Hz, and average= 512). Water suppression (VAPOR sequence, spectral width= 150Hz) achieved with outer volume suppression.

Quantification: In vivo ^{1}H NMR spectra were analyzed using LCModel software. The metabolites that have Cramer-Rao lower bound (CRLB) < 20% were selected for statistical analysis. The unsuppressed water peak was used for water scaling and eddy current correction.

Results:

Shimming resulted in unsuppressed water signal line width (FWHM) of 10~14 Hz. There was a significant change in the ratio of taurine to total creatine in the hippocampus between Gy mice and control mice (taurine/ Cr+PCr= 0.838 ± 0.085 in Gy mice; 1.204 ± 0.070 in control mice, Figure 1-4). The changes in taurine were reversed when Gy mice were bred with CAGSpms line #8 mice to restore spermine synthase activity but not with line #18 (Figure 4).

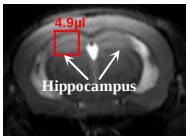

Fig. 1 T_2-weighted RARE image of the mouse brain with the volume of interest in the hippocampus.

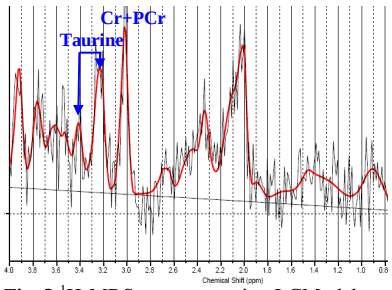

Fig. 2 ^{1}H-MRS spectrum using LCModel curve fit software showing a significant decrease in the taurine peak in the Gy mouse.

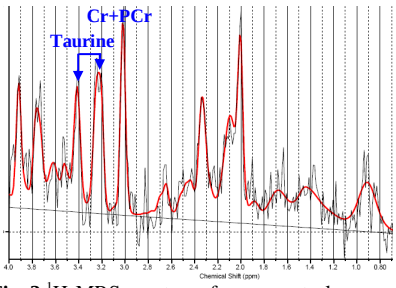

Fig. 3 ^{1}H-MRS spectrum from a control mouse. Peak of Taurine is relatively high compared to the peak from that of Gy mouse.

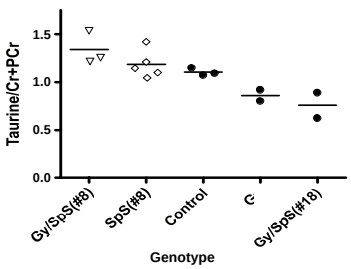

Fig. 4 Taurine percentage change in ippocampus of control, Gy and SpmS mice. (B6C3H & B6D2 background)

Discussion:

In this study, we demonstrated that MRS is a sensitive method to quantify the taurine changes in the brain hippocampus region associated with the SpmS deficiency mouse model. Our data are the first to demonstrate that the spermine deficiency of Gy mice is correlated with a decrease in taurine. This may be related to the fact that methionine is a precursor for both polyamines and sulfur containing amino acids including taurine. Taurine (2-aminoethanesulphonic acid) is one of the most abundant free amino acids in mammals. Taurine has been shown to be involved in many physiological functions. Taurine was found to inhibit Purkinje cell dendrites in the cerebellum and pyramidal cells in the hippocampus by increasing Cl^- conductance. Polyamine may indirectly modulate neuron function by altering taurine level. This result also suggests that MRS may be potentially used as a biomarker for the evaluation of spermine deficiency disease.

Reference:

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