Cerebral Glucose Metabolism in a Transgenic Animal Model of ALS as detected by *In vivo* ^{13}C MRS

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Introduction:

Amyotrophic lateral sclerosis (ALS) is a neuro-degenerative disease characterized by progres-sive muscle weakness, atrophy and degeneration of neurons in brain stem and motor cortex [1]. In addition to these features, a number of mutations have been found in glial glutamate transporters [2]. We investigated mice with the G93A human SOD1 mutation. Expression of this mutant enzyme in vitro results in a loss of mito-chondrial membrane potential and elevated cytosolic calcium concentration [3]. Recently it was observed that oral creatine supplementation improved motor perfor-mance and extended survival in these mice [4]. All these findings suggest that the cerebral energy metabolism may be disturbed in ALS. To characterize effects of these mutations on cerebral glycolytic and TCA cycle metabolism we used *in vivo* and *in vitro* 13C MRS.

Materials and Methods:

Three transgenic ALS mice and three littermates as controls were studied at the age of about 10 weeks. Under Halothane anesthesia, animals received a [1-13C]glucose ip infusion over 2h. *In vivo* 13C spectra of cerebral cortex were acquired at 4.7T with a 13C surface coil (Ø=1cm) and pulse-acquire sequence (ns=300, tr=1s). A double 1H coil was used for 1H broad band decoupling with the WALTZ16 sequence [5]. 5 baseline spectra were recorded prior to glucose infusion. Spectral analysis was done on averages of 2 spectra after gaussian filtering and subtraction of the baseline spectrum. Signal intensities were obtained from fits using the NMR1 software package. Perchloric acid (PCA) extracts of corresponding cortical tissues were investigated by 1H and 13C high resolution MRS at 500MHz. Positional 13C fractional enrichments of selected metabolites were obtained from 1H-observe-13C edited spectra.

Results:

The left side of figure 1 shows a time course of representative *invivo* 13C spectra of 10min intervals of an ALS mouse brain after subtrac-tion of background signal. The uptake of [1-13C] glucose into the brain was the same in both groups as observed by *in vivo* 13C and high resolution MRS. The right side of figure 1 presents the time courses of 13C label incorporation into glutamate C4: the enrichment of glutamate C4 with 13C is higher in transgenic (ALS) animals than in controls (CTRL) (0.26±0.02 vs. 0.22±0.01, $p < 0.05$) although the time courses otherwise look very similar. The higher 13C incorporation into cerebral glutamate in transgenic ALS mice is also supported by high resolution 13C spectra: singlet-to-doublet ratios of glutamate C4 were smaller in ALS than in control (2.22±0.03 vs. 2.75±0.53, $p<0.1$). Additionally, the fractional enrichment of lactate-C3 was higher in ALS animals than in controls (0.278±0.014 vs 0.257±0.011, $p<0.1$).

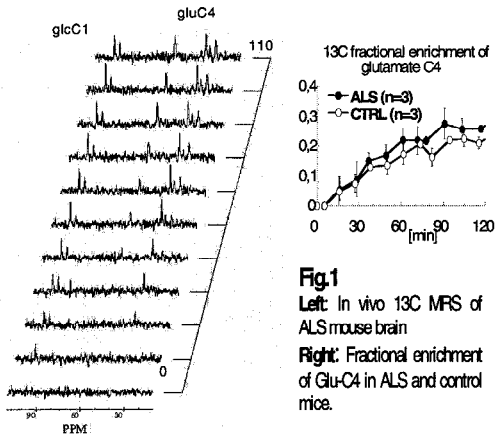

Fig.1
Left: In vivo 13C MRS of ALS mouse brain
Right: Fractional enrichment of Glu-C4 in ALS and control mice.

Conclusion:

The higher incorporation of 13C label into brain metabolites of transgenic ALS animals suggests an increase in brain energy metabolism in these animals. Metabolic fluxes through glycolysis as well as TCA cycle seem to be increased since the fractional enrichments of both lactate-C3 and glutamate-C4 are higher in ALS as compared to their littermates. This is in agreement with the hypothesis of losses of mitochondrial membrane potential which would have to be compensated by increased mitochondrial electron transport and therefore give rise to higher energy demand of cerebral tissue. Alternatively, the differences in 13C enrichment of lactate-C3 and glutamate-C4 may also be explained by different substrate selection at the level of glycolysis: in healthy control animals another endogenous, not 13C enriched, carbon source may contribute to glycolysis and/or to the lactate pool. Unfortunately, the small brain size of mice (which leads to reduced sensitivity and temporal sampling) precludes accurate metabolic modeling from data collected at 4.7T at present. Nonetheless, the abnormalities in glutamate metabolism detected are consistent with data concerning glutamate abnormalities in these mice and in humans [2,6], and point the way towards future studies in humans with much higher sensitivity.

References:

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