Glial-specific inhibition of TCA cycle in rat, by 1H MRS and 13C MRS

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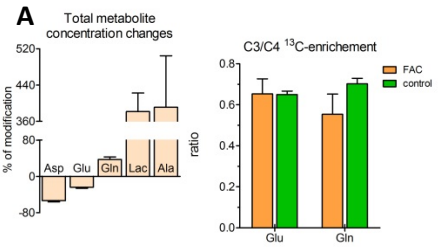
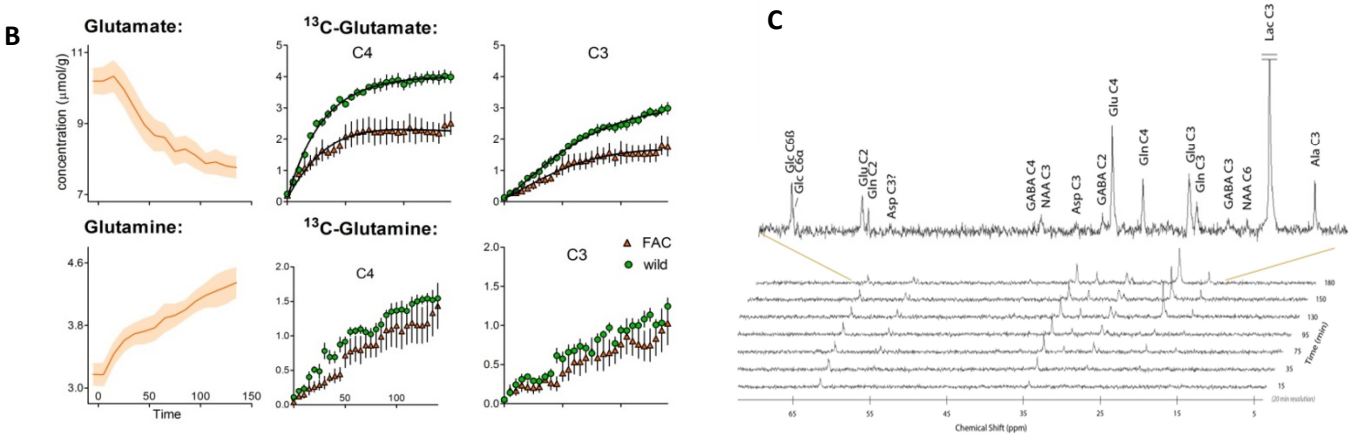
PURPOSE: Cerebral metabolism is driven by glia-neuron interactions and composed of at least two TCA cycle kinetics. While glycolysis is mainly taking place in glial cells, the neuronal oxidative pathway becomes more important. Intercellular exchanges such as neurotransmitter cycling and lactate shuttle are essential for cerebral activity. Using ^{13}C nuclear magnetic resonance spectroscopy *in vivo* and enriched ^{13}C-labeled substrates, in particular glucose, metabolic fluxes can be calculated for different cell types. In this study, we aimed at measuring changes in neurochemical profile upon glial-specific inhibition of the TCA cycle induced by fluoroacetate administration (1,2,3) under hyperglycemia by ^{1}H MRS *in vivo*. In addition, we evaluated the brain-compartmentalized metabolism adaptation to impaired mitochondrial oxidation following ^{13}C-incorporation from glucose into brain amino acids.

METHODS: Male Sprague-Dawley rats (220-250g) under α-chloralose anesthesia and mechanically ventilated (30% O_2 in air, bolus of 80 mg/kg, then 26.7 mg/kg/h) were scanned at 14.1 T with a homemade single loop ^{13}C, ^{1}H quadrature surface coil. The voxel of interest (VOI) was 112 µl, covering cortex and hippocampus. After shimming with FASTMAP, spectra were acquired with STEAM and DEPT for ^{1}H and ^{13}C MRS, respectively. After 10 min baseline, fluoroacetate (FAC) was administered (9 mg/kg) and 20 min later, [1,6-^{13}C] glucose was infused as previously described (7). Heart rate and blood pressure were continuously monitored. Arterial P_aCO_2, P_aO_2, pH, glucose and lactate were measured every 15-20 min. Spectra quantification was performed using LCModel. ^{13}C curves were fitted using a mathematical model as previously described (9); subsequently, a Levenberg–Marquardt algorithm was used for non-linear regression. Reliability of determined fluxes was evaluated by Monte-Carlo analysis.

RESULTS: Through ^{1}H MRS, 21 cerebral metabolites were quantified in 6 rats. 135 minutes following the fluoroacetate injection, glutamate (-24±2%, P<0.001) and aspartate (-53±3%, P<0.001) decrease whereas glutamine (+38±5%, P<0.01), glucose (+297±84%, P<0.001), lactate (+381±40%, P<0.001), and alanine (+391±113%, P<0.001) increase. In [1,6-^{13}C]-glucose infusion experiments, the C3/C4 enrichment ratio was lower in glutamine (0.55±0.09) than glutamate (0.65±0.07) during fluoroacetate treatment. In contrast, untreated animals exhibited practically equal enrichment ratios (gln: 0.70 ±0.07 glu: 0.65±0.04). Assuming a one compartment model of brain metabolism (9), V_{phd} representing TCA cycle rate were 0.40±0.01 for control and 0.35±0.01 for treated animals.



DISCUSSION: Our results are in general agreement with the reported literature (3,4,5), except for the glutamine concentration. Note that in contrast to previous experiments, glucose was continuously infused here; the hence increased availability of energy may have induced increased ammonia detoxification by glutamine synthetase, resulting in the observed glutamine accumulation. Indeed, nitrogen balance between alanine, aspartate, glutamate and glutamine was maintained as the total concentration of these four amino acids remained constant. In ^{13}C MRS experiments, C3/C4 ratios suggested that glutamate pool is generated by faster TCA cycle than glutamine after fluoroacetate exposure, consistent with the inhibition of the glial TCA cycle, the compartment where most glutamine resides. In contrast, the neuronal TCA cycle was poorly influenced by fluoroacetate administration. In conclusion, disruption of ammonia homeostasis by inhibition of glial oxidative metabolism may be compensated by glutamine accumulation through increased energy supply. This may avoid neuronal metabolic dysfunction, which results in unaffected glutamate turnover.



A:Histograms, **on the left** ; percent of increment of total changing metabolite concentration in brain (n=6), **on the right** ; C3/C4 ratio measured at 140min in ^{13}C-enriched metabolite (n=6,5) **B : on the left,** changes in neurochemical profile upon glial-specific inhibition of the TCA cycle of glutamate and glutamine by ^{1}H MRS quantification (n=6). **on the right,** ^{13}C-labeled concentration time curves of glutamate and glutamine enriched at positions C4 and C3 in wild type (n=6) and fluoroacetate treated rats (n=5) of [1,6-^{13}C]glucose infusion. Glutamate concentration curves are fitted by a mathematic model of neuronal TCA cycle (9) . **B:** In vivo ^{13}C MRS spectra evolution of rat brain under fluoroacetate at 14.1T of [1,6-^{13}C]glucose in 112µl of volume of interest.

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