Objectives:
Glutamine synthetase is a critical step in the glutamate-glutamine cycle, the major mechanism of glutamate neurotransmission and is implicated in the mechanism of ammonia toxicity. 15N MRS is an alternative approach to 13C MRS in studying glutamate-glutamine metabolism. 15N MRS studies allow to measure an apparent glutamine synthesis rate (Vsyn) which reflects a combination of the glutamate-glutamine cycle activity (Vnt) and net glutamine accumulation. The net glutamine synthesis (Vsyn-Vnt) can be directly measured from 1H NMR. Therefore, the aim of this study was to perform in vivo localized 1H MRS interleaved with 15N MRS to directly measure the net glutamine synthesis rate and the apparent glutamine synthesis rate under 15N labeled ammonia infusion in the rat brain, respectively.

Methods:
1H and 15N MRS data were acquired interleaved on a 9.4T system (Varian/Magnex Scientific) using 5 rats. 15NH4Cl solution was infused continuously into the femoral vein for up to 10h (4.5mmol/h/kg) (1). The plasma ammonia concentration was increased to 0.95±0.08mmol/l (Analox GM7 analyzer). 1H spectra were acquired and quantified as described previously (2). 15N unlocalized and localized spectra were acquired using the SIRENE sequence (3); and quantified using AMARES and an external reference method (4). The metabolic model used to analyze the total Gln and 5-15N labeled Gln time courses is shown on Fig 1a.

Results:
Glutamine concentration increased from 2.5±0.3mmol/kg to 15±3.3mmol/kg whereas the total glutamate concentrations remained unchanged (Fig. 1b). The linear fit of the time-evolution of the total Gln from the 1H spectra gave the net synthesis flux (Vsyn-Vnt), which was 0.021±0.006µmol/min/g (Fig. 1d). The 5-15N Gln peak (-271ppm) was visible in the first and all subsequent scans, whereas the 2-15N Gln/Glu peak (-342ppm) appeared after ~1.5h (Fig. 1c). From the in vivo 5-15N Gln time course, Vsyn=0.29±0.1µmol/min/g and a plasma NH3 fractional enrichment of 71±6% were calculated. Vnt was 0.26±0.1µmol/min/g, obtained assuming a negligible Gln efflux (5). Vsyn and Vnt were within the range of 13C NMR measurements (6).
Conclusion:
The combination of $^1$H and $^{15}$N NMR allowed for the first time a direct and localized measurement of Vnt and apparent glutamine synthesis rate. Vnt is approximately one order of magnitude faster than the net glutamine accumulation.

References:

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