MEASUREMENT OF GLUTAMINE SYNTHESIS RATE IN THE HYPERAMMONAEMIC RAT BRAIN USING IN VIVO $^1$H AND $^{15}$N MRS

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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. $^{15}$N MRS is an alternative approach to $^{13}$C MRS in studying glutamate-glutamine metabolism. Moreover, the incorporation of $^{15}$N into [5-15N]Gln allows to measure glutamine synthetase activity (Vsyn) directly and can provide a more straightforward interpretation than $^{13}$C studies. Vsyn reflects a combination of the glutamate-glutamine cycle activity (Vnt) and net glutamine accumulation (Vsyn-Vnt). The net glutamine synthesis can be directly measured from $^1$H NMR. The aim of this study was to perform in vivo localized $^1$H MRS interleaved with $^{15}$N MRS to directly measure the net glutamine synthesis rate and the apparent glutamine synthesis rate under $^{15}$N labeled ammonia infusion in the rat brain, respectively.

Methods:
$^1$H and $^{15}$N MRS data were acquired interleaved on a 9.4T system (Varian/Magnex Scientific) using 8 rats. $^{15}$NH$_4$Cl solution was infused continuously into the femoral vein for up to 10h (4.5mmol/h/kg) (1). $^1$H spectra were acquired and quantified as described previously (2). $^{15}$N unlocalized and localized spectra were acquired using the SIRENE sequence (3); and quantified using AMARES and an external reference method (4).

Results and Discussion:
Glutamine concentration increased from 2.5±0.3mmol/kg to 15±3.3mmol/kg (Fig. 1). The linear fit of the time-evolution of the total Gln from the $^1$H spectra gave the net synthesis Vsyn-Vnt=0.023±0.006µmol/min/g (Fig. 2). The 5-$^{15}$N Gln peak (-271ppm) was visible in the first and all subsequent scans, whereas the 2-$^{15}$N Gln/Glu peak (-342ppm) appeared after ~1.5h (Fig. 3). From the in vivo 5-$^{15}$N Gln time course, Vsyn=0.26±0.02µmol/min/g and a plasma NH$_3$ fractional enrichment of 71±6% were calculated. Vnt was 0.24±0.05µmol/min/g, obtained assuming a negligible Gln efflux (5). While Vsyn and Vnt were higher that previous unlocalized $^{15}$N NMR studies, they are within the range of $^{13}$C NMR measurements (6). The combination of 1H and 15N NMR allowed for the first time a direct and localized measurement of Vnt, net glutamine accumulation and apparent glutamine synthesis rate.

References:

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