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. $^{15}NH_4Cl$ 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.

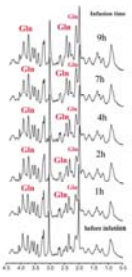

Fig 1 : One series of in vivo ^{1}H spectra acquired at 9.4T in the rat brain.

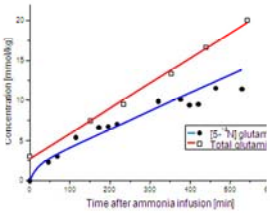

Fig 2 : The time courses and corresponding fits of total Gln at 5-^{15}N Gln from 1 rat.

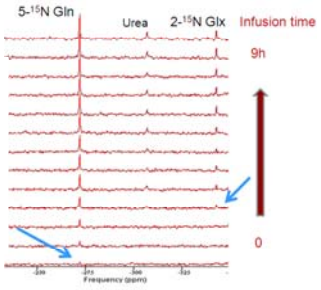

Fig 3 : One series of in vivo unlocalized ^{15}N spectra acquired at 9.4T in the rat brain at different time points.

References:

[1] KanamoriK et al., NMRBiomed 1993;6:21. [2] MlynarikV et al., JMagnReson 2008;194:163. [3] ChoiIY et al., MagnResonMed 2000 ;44 :387 [4] GruetterR, et al., MagnResonMed 1991;20:327 [5] KanamoriK et al., BiochemJ 1993 ;293 :461. [6] SibsonNR et al., J Neurochem 2001; 76:975

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