

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

C Cudalbu, B Lanz, F Morgenthaler, V Mlynárik and R Gruetter

Laboratory for Functional and Metabolic Imaging (LIFMET), Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland.

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- ^{15}N]Gln allows to measure glutamine synthetase activity (V_{syn}) directly and can provide a more straightforward interpretation than ^{13}C studies. V_{syn} reflects a combination of the glutamate-glutamine cycle activity (V_{nt}) and net glutamine accumulation ($V_{\text{syn}}-V_{\text{nt}}$). The net glutamine synthesis can be directly measured from ^1H NMR. The aim of this study was to perform in vivo localized ^1H 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:

^1H and ^{15}N MRS data were acquired interleaved on a 9.4T system (Varian/Magnex Scientific) using 8 rats. $^{15}\text{NH}_4\text{Cl}$ solution was infused continuously into the femoral vein for up to 10h (4.5mmol/h/kg) (1). ^1H 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 $2.5\pm 0.3\text{mmol/kg}$ to $15\pm 3.3\text{mmol/kg}$ (Fig. 1). The linear fit of the time-evolution of the total Gln from the ^1H spectra gave the net synthesis $V_{\text{syn}}-V_{\text{nt}}=0.023\pm 0.006\mu\text{mol/min/g}$ (Fig. 2). The 5- ^{15}N Gln peak (-271ppm) was visible in the first and all subsequent scans, whereas the 2- ^{15}N Gln/Glx peak (-342ppm) appeared after $\sim 1.5\text{h}$ (Fig. 3). From the in vivo 5- ^{15}N Gln time course, $V_{\text{syn}}=0.26\pm 0.02\mu\text{mol/min/g}$ and a plasma NH_3 fractional enrichment of $71\pm 6\%$ were calculated. V_{nt} was $0.24\pm 0.05\mu\text{mol/min/g}$, obtained assuming a negligible Gln efflux (5). While V_{syn} and V_{nt} were higher than previous unlocalized ^{15}N NMR studies, they are within the range of ^{13}C NMR measurements (6). The combination of ^1H and ^{15}N NMR allowed for the first time a direct and localized measurement of V_{nt} , net glutamine accumulation and apparent glutamine synthesis rate.

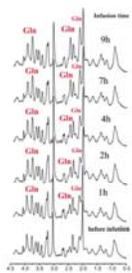


Fig 1 : One series of in vivo ^1H spectra acquired at 9.4T in the rat brain.

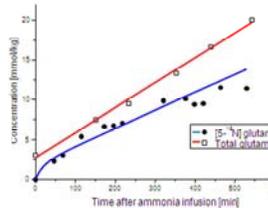


Fig 2 : The time courses and corresponding fits of total Gln at 5- ^{15}N Gln from 1 rat.

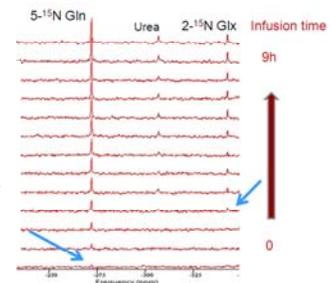


Fig 3 : One series of in vivo unlocalized ^{15}N spectra acquired at 9.4T in the rat brain at different time points.

References:

- [1] Kanamori K et al., NMR Biomed 1993;6:21. [2] Mlynarik V et al., J Magn Reson 2008;194:163. [3] Choi I Y et al., Magn Reson Med 2000;44:387 [4] Gruetter R, et al., Magn Reson Med 1991;20:327 [5] Kanamori K et al., Biochem J 1993;293:461. [6] Sibson N R et al., J Neurochem 2001;76:975

This study was supported by Centre d'Imagerie BioMédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV, EPFL and the Leenaards and Jeantet Foundations and EU Grant No. MRTN-CT-2006-035801