In vivo simultaneous measurement of glutamine synthetase and glutamate dehydrogenase activity in the hyperammonemic rat brain using localized ^{1}H and ^{15}N MRS

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Introduction:

Glutamine synthetase (GS) in brain is predominantly localized in the astrocytes and plays a major role in the detoxification of brain ammonia by converting ammonia and glutamate (Glu) in glutamine (Gln). In addition, some de novo synthesis of Glu from glucose is required to maintain the neurotransmitter pool. Glutamate-dehydrogenase (GDH), along with aspartate aminotransferase, is an enzyme involved in the homeostasis of the brain Glu concentration. ^{15}N MRS is an alternative approach to ^{13}C MRS in studying glutamate-glutamine metabolism and can provide a more straightforward interpretation. Previous ^{15}N MRS studies focused on the incorporation of ^{15}N of ammonia into [5-^{15}N]Gln and measured the apparent glutamine synthetase activity (Vsyn) (1-4). The incorporation of ^{15}N into [2-^{15}N]Gln+Glu may provide further insight into the net glutamate dehydrogenase activity (V_{GDH}). Therefore, for the present study we developed a new pulse sequence in order to simultaneously measure [5-^{15}N]Gln and [2-^{15}N]Gln+Glu for a direct measurement of the net glutamine synthesis rate (Vsyn-Vnt), Vsyn and V_{GDH} under ^{15}N-labeled ammonia infusion in the rat brain, using in vivo localized ^{15}N MRS interleaved with in vivo ^{1}H MRS. In addition, we performed a direct absolute quantification of total Gln, 5-^{15}N Gln and 2-^{15}N Glu+Gln in the same experiment.

Methods:

Six SD rats (300-350g) were fasted overnight. The femoral artery and vein were catheterized for blood sampling as well as ^{15}N ammonium chloride (99%-enriched) and α-chloralose infusion. ^{15}N ammonium chloride was infused continuously (4.5mmol/h/kg) for up to 9-10h (3,4). All the ^{1}H and ^{15}N MRS data were acquired interleaved on a 9.4T system (Varian/Magnex Scientific) using a home-built quadrature ^{1}H coil combined with a single 5-loop 10 mm ^{15}N coil. The ^{1}H spectra were acquired using an ultra-short-TE localized SPECIAL spectroscopy sequence (TE=2.8ms, TR=4s, 128 scans, VOI=6x8x8mm^3) (5). Shimming was performed with FASTMAP. The ^{15}N spectra were acquired using the SIRENE sequence (6). Due to the large chemical shift difference between 5-^{15}N Gln and 2-^{15}N Glu+Gln (~70ppm) the two ^{15}N signals were acquired separately in an interleaved mode using two adiabatic excitation pulses with opposite frequency modulations (Fig. 1). Unlocalized spectra were acquired in the first hours of infusion (256 scans, TR=5s), followed by a localized spectrum (VOI=6x8x8mm^3, 256 averages) used for quantification. ^{1}H spectra were quantified using LCModel. The ^{15}N spectra were quantified using AMARES and an external reference method (7). The metabolic model used is shown on Fig 1. A neuroglial two-compartment model, assuming negligible efflux from the Gln pool compared to Vsyn (1), was fitted simultaneously to the total Gln, 5-^{15}N Gln and 2-^{15}N Glu+Gln curves and values for (Vsyn-Vnt), Vsyn and V_{GDH} were obtained.

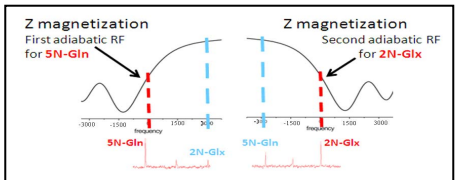

Fig 1: Evolution of Z magnetization for the 2 adiabatic RF pulses used in the new pulse sequence for exciting separately 5-^{15}N Gln and 2-^{15}N Glu+Gln.

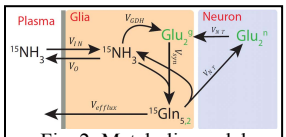

Fig. 2: Metabolic model

Results and Discussion:

Fig. 3 shows a series of in vivo unlocalized ^{15}N spectra acquired in the rat brain during infusion. 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) started to appear from the second scan. Compared with the one RF excitation SIRENE sequence, the new pulse sequence using the 2 adiabatic RF excitation pulses allowed the detection of 2-^{15}N Gln+Glu peak approx 50 min earlier due to a better excitation and an increased SNR. The initial total Gln (0) concentration was 2.5±0.3 mmol/kg$_{ww}$ and increased to 17.7±1.4mmol/kg$_{ww}$ at the end of the infusion, which was in the range of previous biochemical studies (1-3). All other metabolites remained unchanged during the experiment. The time courses of total Gln, 5-^{15}N Gln and 2-^{15}N Gln+Glu were highly reproducible in all six rats. The application of the model to the in vivo data showed an excellent fit (Fig. 4). Based on the model presented in Fig. 1 we obtained from the ^{1}H data a net synthesis flux (Vsyn-Vnt) of 0.035±0.001µmol/min/g. By fitting the in vivo 5-^{15}N Gln and 2-^{15}N Glu+Gln time courses, Vsyn was 0.24±0.03µmol/min/g, V_{GDH} was 0.030±0.001µmol/min/g and the plasma NH_3 FE was 84±6%. Finally, the apparent neurotransmission rate, Vnt, was 0.21±0.03µmol/min/g.

In order to maintain a constant Glu concentration with increasing Gln, glutamate needs to be synthesized from TCA intermediates or from aspartate. Previous studies supposed that this replenishment acts primarily on the glial side in hyperammonemic conditions (2). From high resolution measurements on brain extracts we obtained (2N-^{15}NGln)FE/(2N-^{15}NGlu)FE=1.48±0.04, which proves the fact that net glutamate synthesis results from higher glial GDH activity. In addition, our results also showed that $V_{GDH} \approx$ (Vsyn-Vnt), meaning that V_{GDH} is the primary source of Glu replenishment. Since Vsyn and Vnt which are needed to model the exchange between 2N-^{15}N Glu and 2N-^{15}N Gln are obtained from the simultaneous fit, 2-^{15}N Gln+Glu is sufficient for the measurement of V_{GDH}. We conclude that using this new technique it is feasible to combine localized in vivo ^{15}N with ^{1}H MRS for measuring the net glutamine accumulation and V_{GDH} along with Vsyn under ammonia infusion in the in vivo rat brain. This technique allows a robust absolute quantification of total Gln, 5-^{15}N Gln and 2-^{15}N Gln+Glu in the same experiment. Moreover, in contrast to previous studies, the net synthesis flux (Vsyn-Vnt) and V_{GDH} were directly measured.

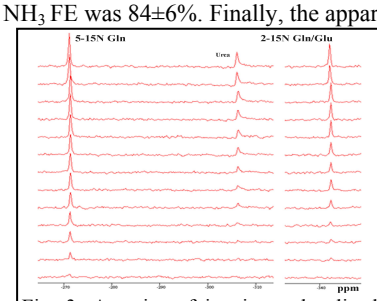

Fig. 3: A series of in vivo unlocalized ^{15}N spectra acquired at 9.4T in the rat brain at different time points for up to 9h.

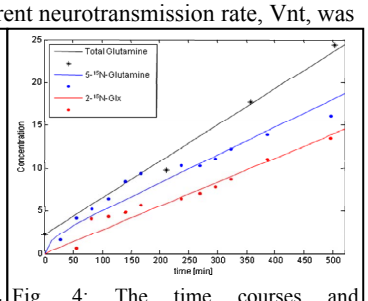

Fig. 4: The time courses and corresponding fits of total Gln, 5-^{15}N Gln and 2-^{15}N Gln+Glu from 1 rat.

References:

[1] Kanamori K et al., Biochem J 1993 ;293 :461. [2]Shen J et al., Dev Neurosci1998 ;20 :434. [3] Kanamori K et al., NMR Biomed 1993;6:21. [4] Cudalbu C et al, Proc Intl Soc Mag Reson Med 17:1578, 2009. [5] Mlynarik V et al., J Magn Reson 2008;194:163. [6] Choi IY et al., Magn Reson Med 2000 ;44 :387 [6] Gruetter R, et al., Magn Reson Med 1991;20:327. **Acknowledgements**: Supported by CIBM of the UNIL, UNIGE, HUG, CHUV, EPFL and the Leenaards and Jeantet Foundations; the EU Grant No. MRTN-CT-2006-035801; and SNF grant No 3100A0-116220.