In vivo simultaneous measurement of glutamine synthetase and glutamate dehydrogenase activity in the hyperammonemic rat brain using localized ¹H and ¹⁵N 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. ¹⁵N MRS is an alternative approach to ¹³C MRS in studying glutamate-glutamine metabolism and can provide a more straightforward interpretation. Previous ¹⁵N MRS studies focused on the incorporation of ¹⁵N of ammonia into [⁵-¹⁵N]Gln and measured the apparent glutamine synthetase activity (Vsyn) (1-4). The incorporation of ¹⁵N into [⁵-¹⁵N]Gln+Glu may provide further insight into the net glutamate dehydrogenase activity (VGDH). Therefore, for the present study we developed a new pulse sequence in order to simultaneously measure [⁵-¹⁵N]Gln and [²⁻¹⁵N]Glu+Glu for a direct measurement of the net glutamine synthesis rate (Vsyn-Vnt), Vsyn and VGDH under ¹⁵N-labeled ammonia infusion in the rat brain, using in vivo localized ¹⁵N MRS interleaved with in vivo ¹H MRS. In addition, we performed a direct absolute quantification of total Gln, ⁵¹⁵N Gln and ²¹⁵N Glu+Glu 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 ¹⁵N ammonium chloride (99%-enriched) and α-chloralose infusion. ¹⁵N ammonium chloride was infused continuously (4.5mmol/h/kg) for up to 9-10h (3,4). All the ¹H and ¹⁵N MRS data were acquired interleaved on a 9.4T system (Varian/Magnex Scientific) using a home-built quadrature ¹H coil combined with a single 5-loop 10 mm ¹⁵N coil. The ¹H spectra were acquired using a short-TE localized SPECIAL spectroscopy sequence (TE=2.8ms, TR=4s, 128 scans, VOI=6x8x8mm³) (5). Shimming was performed with FASTMAP. The ¹⁵N spectra were acquired using the SIReNE sequence (6). Due to the large chemical shift difference between ⁵¹⁵N Gln and ²¹⁵N Glu+Gln (~70ppm) the two ¹⁵N 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=6x8x8mm³, 256 averages) used for quantification. ¹H spectra were quantified using LCModel. The ¹⁵N spectra were quantified using AMARES and an external reference method (7). The metabolic model used is shown in Fig. 1. A neuronal two-compartment model, assuming negligible efflux from the Gln pool compared to Vsyn (1), was fitted simultaneously to the total Gln, ⁵¹⁵N Gln and ²¹⁵N Glu+Gln curves and values for (Vsyn-Vnt), Vsyn and VGDH were obtained.

Results and Discussion:
Fig. 3 shows a series of in vivo unlabeled ¹⁵N spectra acquired in the rat brain during infusion. The ⁵¹⁵N Gln peak (-271ppm) was visible in the first and all subsequent scans, whereas the ²¹⁵N 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 ²¹⁵N 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±.3 mmol/kgww and increased to 17.7±1.4mmol/kgww at the 6-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, ⁵¹⁵N Gln and ²¹⁵N Glu+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 ¹H data a net synthesis flux (Vsyn-Vnt) of 0.035±0.001µmol/min/g. By fitting the in vivo ¹⁵N Gln and ²¹⁵N Glu+Gln time courses, Vsyn was 0.24±0.03µmol/min/g and the plasma NH₃ 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 Glu, glutamate needs to be synthesized from TCA intermediates or from aspartate. Previous studies suggested that this replenishment acts primarily on the glial side in hyperammonemic conditions (2). From high resolution measurements on brain extracts we obtained (2N-¹⁵NGln)FE/(2N-¹⁵NGlu)FE=1.48±0.04, which proves the fact that net glutamate synthesis results from higher glial GDH activity. 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.