in vivo ^{1}H-[^{13}C] NMR Spectroscopy of cerebral GABA turnover

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Introduction

^{1}H-[^{13}C] magnetic resonance spectroscopy (MRS) is a powerful tool to reliably measure the TCA cycle rate and turnover rates of glutamate, glutamine and several other important metabolites[1]. Figure 1 shows a simplified diagram of neuronal and astroglial neurotransmitter metabolism. The black circles indicate the flow of ^{13}C label located at the C_1 position of glucose in the first half turn of the TCA cycle. Carbons at other positions and subsequent turns of the TCA cycle all have a specific labeling pattern. ^{13}C isotopes entering the TCA cycle label 2-oxoglutarate, which is converted to glutamate, establishing a direct metabolic link between energy metabolism and the metabolism of the major excitatory neurotransmitter in the brain[2]. In glutamatergic neurons, the labeled glutamate can be released to interact with post-synaptic targets. The released glutamate is bound to glutamate transporters in the synaptic cleft and transported to astroglial cells limiting excitation. Glutamate is then converted to glutamine, as part of the glutamate-glutamine cycle. In GABAergic neurons, glutamate is converted by GAD to GABA, the major inhibitory neurotransmitter in the brain[2]. GABA turnover *in vivo* has remained below detection threshold in ^{13}C and ^{1}H-[^{13}C] MRS. However, the availability of higher magnetic fields has led to increased spectral resolution and better SNR, lowering this threshold. Therefore, the aim of this study is to measure cerebral GABA metabolism, dynamically and *in vivo* at 9.4 T.

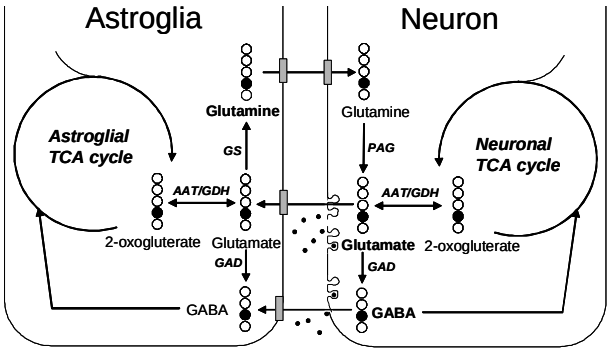

Fig 1: Neuronal and astroglial neurotransmitter metabolism with flow of [1-^{13}C]-glucose label indicated with black circles

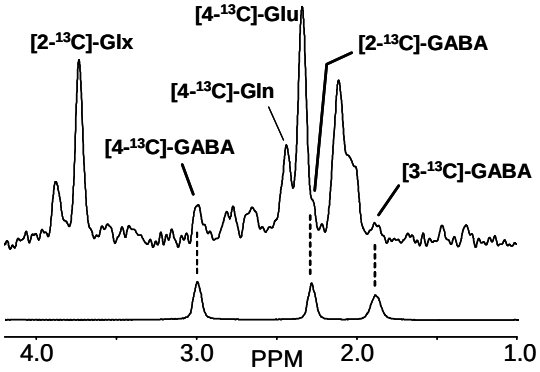

Fig 2: ^{1}H-[^{13}C] MR spectrum 2 hours after infusion of [U-$^{13}C_6$]-glucose (top) and an *in vitro* spectrum of a GABA solution (bottom)

Methods

Male Wistar rats (200 g, n=5) were tracheotomized and ventilated with 0.5% halothane in O_2/N_2O (30/70). A femoral artery and vein were cannulated for monitoring of pCO_2, pO_2 and blood pressure and infusion of [U-$^{13}C_6$]-glucose, according to a previously described protocol[1]. During MR experiments the animals were restrained in a head holder and immobilized with d-tubocurarine chloride. Experiments were performed on a 9.4 T Magnex magnet and Bruker console equipped with a 9 cm diameter gradient coil insert (500 mT/m, 165 µs). A 14 mm diameter surface coil was used for ^{1}H RF pulse excitation and signal reception, while two orthogonal 21 mm-diameter surface coils were used for ^{13}C spectral editing and decoupling. Adiabatic pulses with a bandwidth of 6600 Hz were used for decoupling all metabolites of interest, except for α-H1-glucose, enabling direct quantification of cerebral glucose fractional enrichment. 3-D localization was achieved by a combination of OVS, ISIS and slice-selective excitation (voxel size 180 µl). Adiabatic pulses were used for water suppression[1].

Results

Figure 2 shows a ^{1}H-[^{13}C] spectrum, 2 hours following infusion of [U-$^{13}C_6$]-glucose, after subtraction of the ^{13}C natural abundance contribution. Despite the low concentration of GABA (0.8-1 mM) *in vivo*, the ^{13}C labelled resonances of GABA are clearly visible at 2.28 ppm (H2), 1.89 ppm (H3) and 3.01 ppm (H4). The labelling of GABA was confirmed by comparing the *in vivo* ^{1}H-[^{13}C] spectrum with an *in vitro* spectrum of a GABA solution. The *in vivo* chemical shifts and amplitudes correspond well with the presence of GABA. The turnover of GABA was further confirmed by the sequential labelling of GABA-H2 (within 30 min) followed by GABA-H3 and GABA-H4. The turnover curves of GABA, glutamate and glutamine, are modelled with a 4-compartment metabolic model to give absolute metabolic fluxes, which relate cerebral energy metabolism with inhibitory and excitatory neurotransmission.

Discussion

We show that it is possible to quantify GABA turnover *in vivo* in the rat brain with ^{1}H-[^{13}C] MRS at 9.4 T from a relatively small volume, enabling simultaneous and non-invasive monitoring of inhibitory and excitatory neurotransmitter metabolism as well as TCA cycle rate. This provides us with the opportunity to investigate the contribution of inhibitory and excitatory neurotransmission to the stoichiometric coupling of energy metabolism and neuronal activation[3]. The possibility to measure GABA turnover also has some interesting clinical implications, since aberrant GABA concentrations and metabolism have been shown to contribute to pathological conditions such as anxiety disorders and epilepsy[2]. The latter is especially interesting, since glutamatergic neurotransmission has been shown to be compromised as well[2]. In the future, our understanding and assessment of GABAergic metabolism can be further improved by infusing ^{13}C labeled compounds that target metabolic pathways more specifically, e.g. [2-^{13}C]-acetate, which labels astroglia, and by using segmentation techniques for discrimination of grey and white matter metabolism.

Acknowledgements

This research was supported by NIH grant RO1-EB002097, DK-27121, NS-34813 and by the National Epilepsy Foundation, The Netherlands

References

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