Measurement of the GABA/Glutamine cycling rate in the human brain using ^{13}C MRS

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Introduction

Glutamate and GABA are the major excitatory and inhibitory neurotransmitters in the brain. The use of ^{13}C NMR spectroscopy during the infusion of ^{13}C labeled substrates allows the determination of neurotransmitter cycling flux (V_{Cyc}) and oxidative energy synthesis (V_{TCA}) from the turnover of Glutamate and Glutamine [1-6]. Because Gln is a common precursor for both Glu and GABA, it is not possible to separate GABA/Gln from Glu/Gln cycling in a study using [1-^{13}C] glucose alone. A previous ^{13}C study performed in the rat cortex *in vivo* has shown that a three compartment model and a labeling strategy combining data acquired from [2-^{13}C]acetate and [1-^{13}C]glucose infusions allow for the determination of the contribution of GABA to Glu/Gln cycling and energy metabolism [1]. In this study, we evaluated the relative contributions of GABAergic and glutamatergic cycling and oxidation using ^{13}C NMR spectroscopy during infusions of either [2-^{13}C]acetate and [1-^{13}C]glucose on the same human subjects.

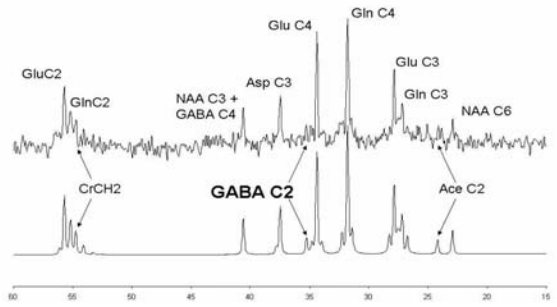

Fig. 1. *LCModel fit of an in vivo ^{13}C NMR spectrum corresponding to the last 40 min averaged over the 5 acetate experiments (3Hz Gaussian filtering, 4-fold zero-filling).*

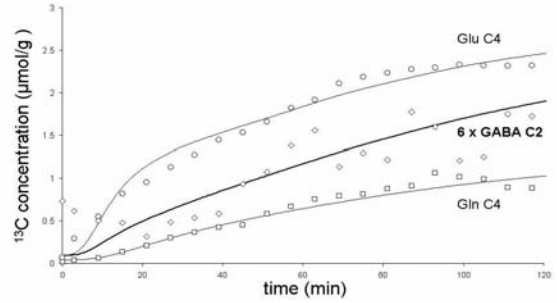

Fig. 2. *Time-courses of Glu C4 (○), Gln C4 (□) and GABA C2 (◊) ^{13}C concentrations measured in one of the 5 glucose experiment and best fits.*

	GABA C2	*Gln C4*	*Gln C3*	*Gln C2*
APE %	*4.7 ± 2.3*	*11.9 ± 1.3*	*5.1 ± 1.3*	*5.6 ± 1.9*
	AspC3	*Glu C4*	*Glu C3*	*Glu C2*
APE %	*12.9 ± 1.5*	*3.9 ± 0.6*	*3.3 ± 0.5*	*4.2 ± 0.8*

Table1. *Steady state APE% from [2-^{13}C]acetate infusion (determined from the sum of the last 40 min averaged over the 5 experiments).*

Materials and Methods

MRS acquisition. Both [2-^{13}C]acetate and [1-^{13}C]glucose experiments were conducted on five young healthy volunteers (aged 24 ± 2 years, BMI 23.5 ± 2.0 kg/m^2, three men and two women). Subjects lay supine in a 4.0 T whole-body magnet (Bruker Instruments, Billerica, MA) with the head lying on top of one 8.5-cm-diameter ^{13}C circular coil and two ^{1}H quadrature coils for ^{1}H acquisition and decoupling. After tuning, acquisition of scout images, shimming with the FASTERMAP procedure [7], and calibration of the decoupling power, ^{13}C MRS spectra were acquired with polarization transfer [3] from a 50x40x45 mm^3 volume located in the occipital-parietal lobe before and during infusion of either [1-^{13}C]glucose or [2-^{13}C]acetate (TR=2500ms, 128 transients). During the acquisition, blood samples were collected every 5-10 minutes to measure glucose, acetate and lactate plasmatic concentration and ^{13}C fractional enrichment.

MRS spectral analysis. The scans were added in running averages of 15 min (i.e., three 5 min blocks). Spectra were analyzed using LCModel 6.1 [8] (Stephen Provencher Inc., Oakville, Ontario, Canada) with modified input parameters and a simulated basis set generated as described by Henry et al. [9]. Concentrations for the different isotopomers of Glutamate, Glutamine, Aspartate and GABA were obtained relatively to the signal of natural abundance NAA assuming a concentration of 11 µmol/g.

Metabolic modeling analysis. The steady states fractional enrichments of Asp C3, GABA C2, Glu C4, C3 and C2, and Gln C4, C3 and C2 were determined from the sum of the last 40 min averaged over the five [2-^{13}C]acetate experiments (Table1). These values were then used to compute the ratios V_{Cyc}/V_{TCAn} for GABAergic and glutamatergic neurons using relationships derived from the analytical solutions of the differential equations that describe the flow of ^{13}C through the Glu/Gln and GABA/Gln cycles at steady-state [1,2,4,6]. The ratios were then used as parameter constraints in fitting a three-compartment metabolic model to the time courses of ^{13}C labeling amino acids from the individual [1-^{13}C]glucose experiments performed in the same conditions. Metabolic modeling was performed using CWave[4].

Results

Using the values in Table 1, we determine V_{Cyc}/V_{TCA} for both GABAergic and glutamatergic neurons to be respectively 0.65 and 0.49. These values match well V_{Cyc}/V_{TCA} ratios reported for previous ^{13}C studies in the rat cortex (GABAergic: 0.63) [1] or in the human brain (glutamatergic: 0.46 and 0.39) [3,6]. The values of V_{TCAn} and V_{Cyc} for both GABAergic and glutamatergic neurons as well as V_{TCAa} were determined by the fitting of the metabolic model. The best fits from an individual experiment are shown in Figure 2 for time courses of GluC4, GlnC4 and GABAC2 ^{13}C concentrations. TCA cycle flux in GABAergic and glutamatergic neurons was 10 % and 90 % of V_{TCAn} total respectively while neurotransmitter cycling was 8 % and 92 % of $V_{Cyc(Total)}$ respectively. These values are consistent with studies in the rat cortex [1].

Conclusion

Despite the critical importance of inhibitory processes, to date there have been no measurements in humans of GABA neurotransmitter release and recycling. The use of ^{13}C MRS to measure GABA and Glutamate labeling during [2-^{13}C]acetate and [1-^{13}C]glucose infusions in combination with a 3 compartment model allowed the first measurement of GABA neurotransmitter cycling and the GABAergic TCA cycle in human brain. Although, GABAergic neurotransmission contributes less than glutamatergic neurotransmission to total cycling, the present study shows that it forms an appreciable flow rate. These results may form the basis for applications to directly study alterations in the GABA/Gln cycle in disease such as depression and epilepsy.

Acknowledgments

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