Simultaneous Measurement of Neuronal and Glial Metabolism in Rat Brain *In Vivo* Using Co-infusion of [1,6-$^{13}C_2$]Glucose and [1,2-$^{13}C_2$]Acetate

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Introduction

^{13}C NMR spectroscopy combined with infusion of ^{13}C-enriched substrates (e.g glucose or acetate) is a powerful tool to study compartmentalized neuronal-glial metabolism in the brain [1-3]. Co-infusion of [1-^{13}C]glucose and [1,2-$^{13}C_2$]acetate has been used to discriminate glial and neuronal metabolism in brain extracts based on the different isotopomer pattern obtained in glutamate and glutamine [1,4]. [1-^{13}C]glucose leads to the formation of [4-^{13}C]glutamate and [4-^{13}C]glutamine (singlets) whereas [1,2-$^{13}C_2$]acetate yields [4,5-$^{13}C_2$]glutamate and [4,5-$^{13}C_2$]glutamine (doublets, J_{45} ~52 Hz).The aim of the present study was to demonstrate the feasibility of this approach *in vivo*.

Methods

Overnight fasted male Sprague-Dawley rats (n=3) were infused (i.v.) with 1.6 M [1,2-$^{13}C_2$]acetate and 1.0 M [1,6-$^{13}C_2$]glucose under isoflurane anesthesia for 3 hours. *In vivo* ^{1}H-localized ^{13}C NMR spectra from the brain (VOI of 400µl) were measured on a 9.4T horizontal magnet using a semi-adiabatic DEPT sequence [5]. *In vivo* spectra were analyzed automatically using LCModel [6]. Physiological condition was monitored and maintained throughout the experiment. Blood samples were collected to determine plasma glucose and acetate concentration and enrichment using high-resolution NMR.

Results

An *in vivo* ^{13}C NMR spectrum measured in the rat brain after infusion of ^{13}C-labeled substrates showed ^{13}C labeling of numerous resonances in multiple metabolites such as glutamate, glutamine or GABA (Fig. 1). The fitted spectrum obtained with LCModel closely matched the *in vivo* spectrum.

Time courses of ^{13}C concentration of individual isotopomers (singlets and multiplets) of glutamate and glutamine C4 resonances were measured *in vivo* with a temporal resolution of 11 min (Fig. 2). The time course of glutamine C4 showed a large contribution from the doublet D45 compared to its singlet, indicating that a large fraction of the synthesized glutamine came from glial metabolism of [1,2-$^{13}C_2$]acetate. In contrast, the glutamate C4 time course was dominated by a singlet, indicating that most glutamate is synthesized from [1,6-$^{13}C_2$]glucose metabolism in neurons. These directly reflected the fact that glucose and acetate are metabolized in different metabolic compartments in the brain. The isotopic fractional enrichment in glutamate C4 and glutamine C4 was similar at 3 hours (40 ± 2 % in both cases). ^{13}C concentration time courses of C2 and C3 glutamate and glutamine were also measured *in vivo* (data not shown).

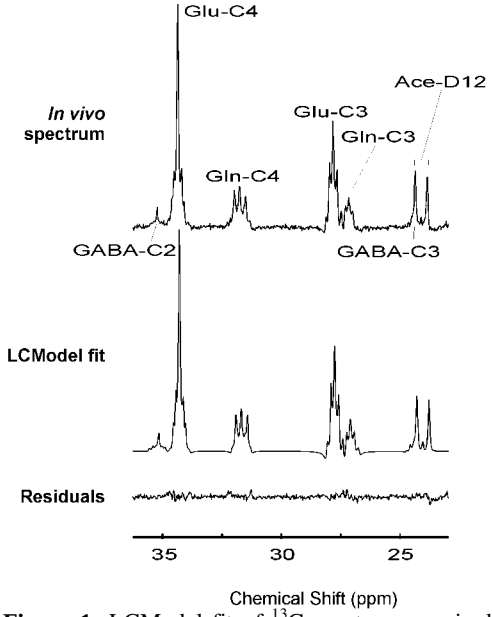

Figure 1: LCModel fit of ^{13}C spectrum acquired during 3 hours of co-infusion of acetate and glucose. From top to bottom: *in vivo* spectrum, fitted spectrum and residuals.

Discussion and Conclusion

This study has shown the feasibility of measuring dynamic time courses of individual ^{13}C isotopomers of glutamate and glutamine in rat brain *in vivo* during simultaneous infusion of [1,6-$^{13}C_2$]glucose and [1,2-$^{13}C_2$]acetate. We expect that dynamic metabolic modeling of these ^{13}C time courses will lead to more precise and reliable determination of metabolic fluxes in the brain, particularly the glutamate-glutamine cycling rate V_{NT}.

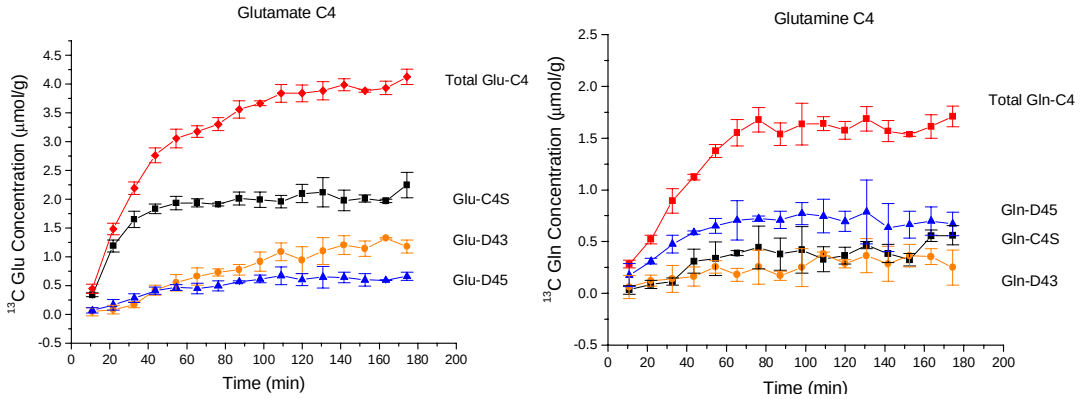

Figure 2: Dynamic *in vivo* ^{13}C concentration time courses of glutamate C4 and glutamine C4 isotopomers in the rat brain during co-infusion of [1,6-$^{13}C_2$]glucose and [1,2-$^{13}C_2$]acetate. Time resolution is 11 min (mean from 3 animals).

References

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Acknowledgments

This work was supported by NIH P41 RR008079, P30 NS057091, R01 NS038672 and the Keck Foundation.