Dynamic Metabolic Modeling of [2-13C]Acetate Metabolism in the Rat Brain

A. A. Shestov[1], D. K. Deelchand[1], and P-G. Henry[1]
[1]Radiology, University of Minnesota Medical School, Minneapolis, MN, United States

Introduction

Carbon-13 MRS combined with metabolic modeling allows measurement of metabolic rates *in vivo*. Most ^{13}C metabolic modeling studies have been performed using ^{13}C-glucose as the infused substrate. Acetate, a glial-specific substrate, is an attractive alternative to glucose for the study of neuronal-glial interactions [1,2]. The goal of present study were: 1) to determine kinetic parameters for acetate transport and utilization; and 2) to perform *dynamic* metabolic modeling of glutamate and glutamine ^{13}C turnover curves obtained during ^{13}C-acetate infusion with a two-compartment neuronal-glial model.

Methods

In vivo ^{13}C glutamate and glutamine ^{13}C labeling time courses were measured during infusion of [2-^{13}C] acetate in rat brain under morphine anesthesia at 9.4 T [3]. The kinetics parameters for acetate transport through the BBB were obtained with a reversible non-steady-state Michaelis-Menten model. V_{max} and K_M for acetate transport and utilization in the brain were determined by simultaneously fitting the two following curves: 1) the time course of $[Ace]_{brain}$ measured in animals (where the ^{13}C-acetate level in plasma and brain was constant) using $[Ace]_{plasma}$ as input function and 2) the relationship $[Ace]_{brain} = f([Ace]_{plasma})$ obtained by plotting the values of $[Ace]_{plasma}$ and $[Ace]_{brain}$ at steady-state for each animal. Monte-Carlo simulations were conducted to verify the stability and precision of estimated parameters.

Using the kinetics information of acetate transport and uptake, *dynamic* modeling of glutamate and glutamine ^{13}C labeling curves acquired during ^{13}C-acetate infusion was performed using a two-compartment neuronal-glial model [4]. Least-square fittings were performed in Matlab.

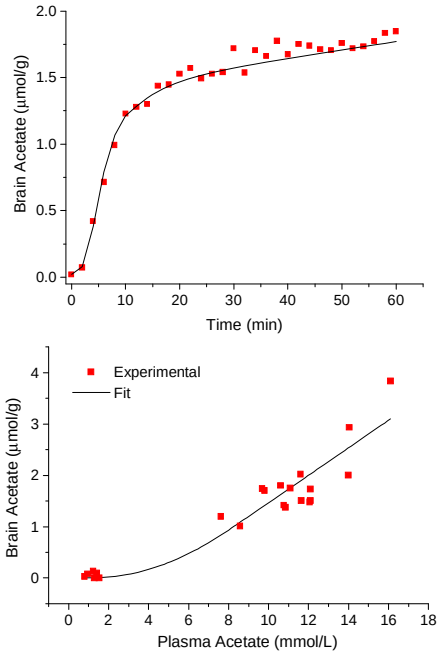

Figure 1: Brain acetate vs. time and steady-state curve Ace(brain) vs. Ace(blood) Solid lines represent best fit obtained by simultaneous fitting of time course and steady state data (two curves).

Results and Discussion

Fitted values for transport and uptake kinetics were: V^{tr}_{max}= 0.96 ± 0.18 µmol/g/min and K^{tr}_M= 4.2 ± 1.8 mM for acetate transport through the BBB and V^{ut}_{max}=0.50 ± 0.08 µmol/g/min with K^{ut}_M= 0.01 ± 0.14 mM for acetate utilization through the mitochondrial inner membrane and acetyl-CoA synthetase from acetate. Therefore, at high concentration of plasma acetate, the rate-limiting step for acetate utilization is not transport through the blood-brain barrier, but occurs after entry of acetate into the brain. The steady-state cerebral metabolic rate of acetate (CMR_{ace}) was 0.49 ± 0.08 µmol/g/min (mean ± SD; n = 4).

Metabolic fluxes determined from metabolic modeling of the glutamate and glutamine ^{13}C time courses were (in µmol/g/min): $V_{TCA(n)}$= 0.95 ± 0.22, $V_{TCA(g)}$= 0.21 ± 0.02, V_{PC}= 0.04 ± 0.01, V_X=1.2 ± 0.2 and V_{NT}= 0.15 ± 0.03. These values are in agreement with rates reported in previous studies. Monte-Carlo simulations suggest that the determination of the glial TCA cycle rate $V_{TCA(g)}$ is more precise when using ^{13}C-acetate than when using ^{13}C-glucose (not shown).

Conclusion

1) At high plasma acetate concentration (~2-3 mM and above), acetate metabolism is rate-limited after entry of acetate into the brain rather than by the blood-brain barrier; 2) Dynamic metabolic modeling of glutamate and glutamine ^{13}C turnover curves measured during [2-^{13}C]acetate infusion with a two-compartment neuronal-glial model is feasible and allows determination of compartmentalized metabolic rates.

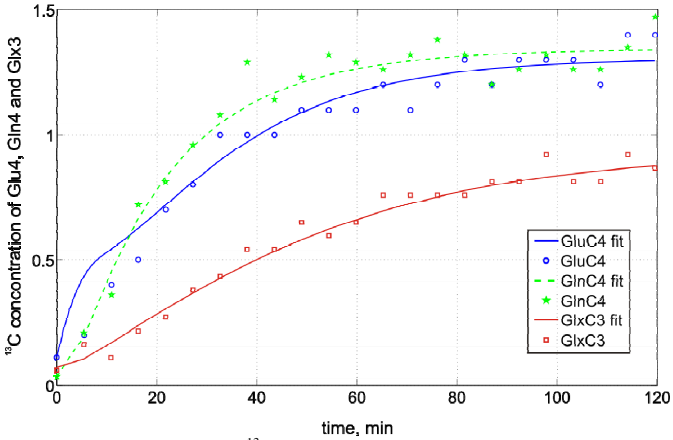

Figure 2: Dynamic *in vivo* ^{13}C concentration time courses of glutamate C4, glutamine C4 and average of glutamate +glutamine at C3 (GlxC3) during infusion of [2-^{13}C]acetate. Lines represent best fit.

References

[1] Bluml et al. NMR Biomed 2002 ; [2] Lebon et al. J Neurosci 2002 [3] Deelchand et al. ISMRM 2006; [4] Gruetter et al . AJP 2001.

Acknowledgments

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