On the reliability of ^{13}C metabolic modeling using two-compartment neuronal-glial models

A. Shestov[1], K. Ugurbil[1], P-G. Henry[1]

[1]Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN, United States

Introduction

^{13}C NMR spectroscopy in conjunction with the infusion of ^{13}C-labeled substrates has emerged as a unique tool to investigate compartmentalized brain metabolism [1]. Analysis of ^{13}C turnover curves using a two-compartment neuronal-glial model of brain metabolism has allowed measurement of metabolic fluxes such as the glial and neuronal TCA cycle rates and the rate of glutamate-glutamine cycling [2,3]. However the robustness of metabolic flux determination using two-compartment models has not been systematically investigated. The goal of this work was to assess the reliability of two-compartment metabolic modeling using Monte-Carlo simulations.

Methods

All simulations were carried out in Matlab (The MathWorks Inc) using a previously published metabolic model [2]. The system of differential equations was solved with the ODE solver in Matlab and least-square fitting was performed using a BFGS minimization algorithm. Six fluxes were left as free parameters in the model: the neuronal and glial TCA cycle rates (Vpdh and Vg), the pyruvate carboxylase flux (Vpc), the rate of exchange between mitochondrial 2-oxoglutarate and cytosol glutamate (Vx), the rate of glutamate-glutamine cycle (Vnt) and the rate of lactate dilution (Vout). For Monte-Carlo simulations, differential equations were solved using the following nominal parameters (in μmol.g^{-1}.min^{-1}): Vpdh=1, Vg=0.1, Vpc=0.1, Vx=1, Vnt=0.3, Vout=0.3 (these data are representative of a "human" data set [2]), to yield simulated time courses for glutamate C4, C3, C2, glutamine C4, C3, C2 and aspartate C23 (average of aspartate C2 and C3). Time courses were fitted after adding Gaussian white noise (standard deviation σ). This procedure was repeated at least 500 times with a different noise realization to yield a statistical distribution for each free parameter in the model [4].

Results and Discussion

Monte-Carlo simulations were performed in the case of a [1-^{13}C]-glucose infusion while varying the following experimental conditions: number of experimental data points per turnover curve (Fig.1), number of fitted time courses (Fig.2) and noise level (Fig.3). The relative standard deviation (SD) increased dramatically as the number of experimental points decreased, especially for Vnt (Fig.1). The relative SD for Vnt was 45% with 50 experimental time points per turnover curve and increased to an extremely high value (30,000%) with 10 experimental time points. The relative SD also increased as the number of fitted curves decreased (Fig. 2). For example, using only 3 curves (Glu C4, Gln C4 and Glx C3) led to much higher relative SD than when using 4 curves (Glu C4, Gln C4, Glu C3, Gln C3) for all fluxes. The relative SD for Vnt increased from 64% with 4 curves fitted to 270% with 3 curves fitted (Fig.2) and to 44,000% with only 2 curves fitted (not shown). Finally, lower signal-to-noise ratio on turnover curves also led to an increase in relative SD of metabolic fluxes (Fig.3). All fluxes became less precise with higher noise, with Vnt showing a sharp increase: the relative SD for Vnt was 45% with σ= 0.2 and 5,800% with σ= 0.3.

Our simulations demonstrate that the determination of Vnt may be unreliable under certain experimental conditions. One way to alleviate this problem is to introduce more constraints in the model. For example, the ratio Vnt/Vpdh can be determined using acetate infusion (acetate being taken up only by glia) and the ratio can be used as an additional constraint in the fitting [5]. Indeed, the confidence interval on Vnt was much narrower (CI = 0.22-0.39) when using the constrained ratio Vnt/Vpdh than when using no constraint (CI =0.08-1.22) (Figure 4).

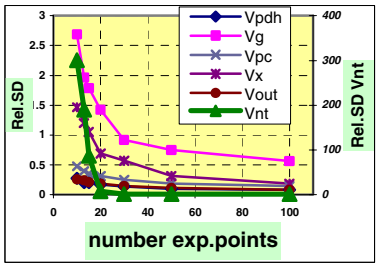

Fig.1. Relative SD on metabolic fluxes (Vpdh, Vg, Vnt, Vx, Vpc, Vout) as a function of the number of data points per turnover curve. (with noise σ = 0.2 μmol.g^{-1} and 7 turnover curves fitted: Glu C4,C3, C2, Gln C4, C3, C2, Asp C23). Relative SD increases sharply as the number of points decreases. Note the different vertical scale for Vnt.

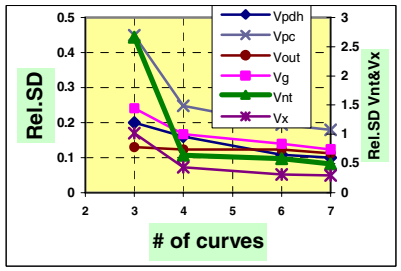

Fig 2. Relative SD on metabolic fluxes (Vpdh, Vg, Vnt, Vx, Vpc, Vout) as a function of the number of turnover curves used in the fitting. (with noise σ= 0.2 μmol.g^{-1} and 50 experimental points per turnover curve). Relative SD increases sharply when less than 4 curves are used.

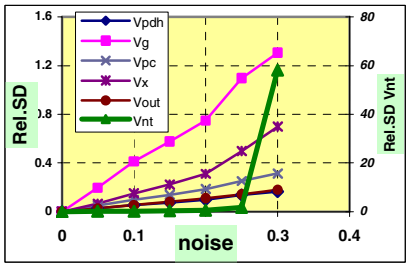

Fig 3. Relative SD on metabolic fluxes (Vpdh, Vg, Vnt, Vx, Vpc, Vout) as a function of the noise level on turnover curves. (with and 7 turnover curves fitted and 50 experimental points per turnover curve). Relative SD increases with higher noise level.

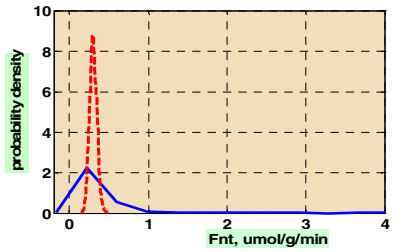

Fig.4. Probability distribution for Vnt with (blue curve) and without (red dashed curve) using a constrained Vnt/Vpdh ratio in the model (7 curves fitted; 20 experimental points per curve; noise level σ= 0.2). The precision of Vnt determination is much improved when using the additional constraint on Vnt/Vpdh.

Conclusion

In conclusion, we show that determination of metabolic fluxes using two-compartment neuronal-glial models may not be reliable at low S/N, with few experimental points, or with few turnover curves. In those cases, additional constraints are needed in the model.

References

1. Sibson, N.R. *et al.*, *PNAS*, 95, 316 (1998).
2. Gruetter, R. *et al.*, *Am. J. Physiol.*, 281, E100 (2001).
3. Shen, J. *et al. Proc. Natl. Acad. Sci. USA* ,96, 8235 (1999)
4. Henry, P.-G. *et al.*, *J. Neurochem.*,. 82, 857 (2002).
5. Lebon, V. *et al.* J. Neurosci., 22,5 (2002).

Acknowledgements

Supported by NIH P41RR08079, R01NS38672, the MIND Institute and the Keck Foundation.