Determination of the Glutamate-Glutamine Cycling Flux Using Two-Compartment Dynamic Metabolic Modeling Is Sensitive to Astroglial Dilution

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Introduction

We examine the certainty of determining the rate of the glutamate-glutamine cycling pathway (V_{cyc}) from [1-^{13}C] glucose infusions. Monte Carlo simulations were performed using the two-compartment model previously published by Shen et al (1), which takes into account the dilution of astroglial glutamine ^{13}C labeling due to exchange with blood glutamine and other metabolic pathways such as fatty acid oxidation in astroglia. The noise level, concentration, temporal resolution, and fractional enrichments were based on a previous ^{13}C MRS studies of human occipital lobe (1). To avoid false minima, which are well-known to plague optimization algorithms such as BFGS or Simplex, simulated annealing was used to derive the best fits. The standard Chi-square (χ^2) statistical analysis of the Monte Carlo fits was also performed to evaluate the goodness-of-fit. We show that using only the glutamate C4 and glutamine C4 ^{13}C turnover curves, V_{cyc} can be reliably extracted from two-compartment modeling of the glutamate neurotransmitter cycling between neurons and astroglia.

Methods

The mean metabolic fluxes from the resting human brain (1) were used (in µmol/g/min): neuronal TCA cycle rate $^{n}V_{TCA}$ = 0.71, glial TCA cycle rate $^{g}V_{TCA}$ = 0.06, glutamate-glutamine cycling rate V_{cyc} = 0.32, anaplerosis rate V_{ana} = 0.04, lactate dilution rate $V_{dil(Lac)}$ = 0.05, glutamine dilution rate $V_{dil(Gln)}$ = 0.14. Metabolic concentrations were as follows (in mM): [Glu] = 9.1, [Gln] = 4.3 as described in ref (1). The metabolic model consisted of two coupled differential equations which were solved numerically, yielding turnover curves of glutamate C4 and glutamine C4 as labeled from [1-^{13}C]glucose. Monte Carlo simulation procedure was carried out as follows: synthetic curves for glutamate C4 and glutamine C4 were first generated by solving the two coupled differential equations. Then, noise of normal distribution with a predefined standard deviation (σ) was added to the synthetic curve. A total of 160 minutes of infusion time was simulated with 32 data points per curve. Two noise levels were investigated, σ = 0.1 µmol/g and 0.2 µmol/g, which spanned the range of noise levels observed within the experimental data sets. A total of 100 synthetic data sets for each simulation (i.e., different noise realization but with the same σ and other conditions) were generated using the Monte Carlo method. Each data set was then fitted using the metabolic model to obtain the best fit values for each of the free metabolic fluxes. All metabolic fluxes were constrained between 0 and 10 µmol/g/min unless specified otherwise. Minimization was performed using the simulated annealing algorithm which is well-suited to finding the global minimum in a multidimensional error space. The goodness-of-fit was analyzed using the standard χ^2 statistics.

Results

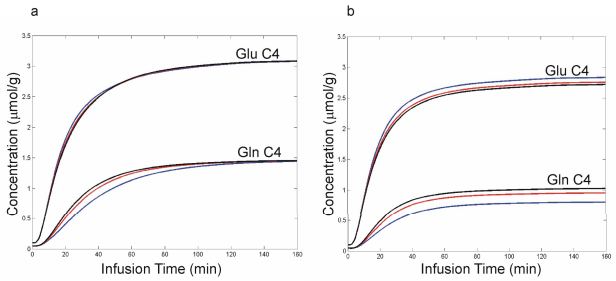
Table 1 summarizes the results of Monte Carlo simulations performed for each predetermined condition (noise level and constraints). With no constraints and a noise level of σ = 0.1 µmol/g a mean V_{cyc} of 0.27 µmol/g/min was calculated with a SD of 37%. At the higher noise level of σ = 0.2 µmol/g a lower value of V_{cyc} (0.16 µmol/g/min) was calculated due to that the fitting tends to calculate a very high value of $^{g}V_{TCA}$. When a constraint was added that $^{g}V_{TCA}$ was less than 0.1 µmol/g, a V_{cyc} of 0.32 µmol/g/min was calculated for both noise levels with a reduced SD. An additional reduction in the SD was obtained by also constraining the value for the net rate of glutamine efflux. Constraining the values for the dilution of the glutamate and glutamine C4 pools further reduced the SD of the calculations. In order to assess the importance of the astroglial dilution flux in the accuracy and precision of the calculations simulations were also carried out with $V_{dil(Gln)}$ forced to be zero. For σ = 0.2 µmol/g, a relative standard deviation of 438% was obtained for V_{cyc}, indicating this flux cannot be reliably determined under the simulated condition ($V_{dil(Gln)}$=0) using two-compartment modeling. A large decline in uncertainty was observed at the noise level of 0.1 µmol/g, but the relative standard deviation of 66% was still very high when $V_{dil(Gln)}$ was forced to be zero.

Table 1. Standard deviation of V_{cyc} determined using Monte Carlo analysis of the two-compartment model with astroglial dilution†

Constraints (µmol/g/min)	Mean V_{cyc} (µmol/g/min)	Rel. SD V_{cyc}¶ (%)	Noise (µmol/g)
None	0.16	34	0.2
	0.27	37	0.1
$^{g}V_{TCA}\leq 0.1$	0.32	37	0.2
	0.32	28	0.1
$^{g}V_{TCA}\leq 0.1$, $V_{efflux}=0.2V_{Gln}$	0.26	28	0.2
	0.26	19	0.1
$V_{dil(Gln)}$§	0.31	34	0.2
	0.33	19	0.1
$V_{dil(Lac)}$§, $V_{dil(Gln)}$§	0.32	29	0.2
	0.32	17	0.1
$^{g}V_{TCA}\leq 0.1$, $V_{dil(Lac)}$§, $V_{dil(Gln)}$§	0.33	28	0.2
	0.33	16	0.1

†Mean χ^2 of all simulations lies in the range of 59.0 - 60.8 with a standard deviation in the range of 10.9 - 11.4. ¶With respect to the nominal V_{cyc} = 0.32 µmol/g/min. The expected mean χ^2 is ~N (no. of data points)-n (no. of free fluxes) with a SD of ~$(2(N-n))^{0.5}$.

Fig. 1 Analysis of the sensitivity of the glutamate C4 and glutamine C4 curves to changes in V_{cyc} (a) $V_{dil(Gln)}$ = 0; (b) $V_{dil(Gln)}$ = 0.14 µmol/g/min.



Discussion

The sensitivity of the glutamine C4 curve to V_{cyc} as revealed by the results in Table 1 can be easily understood by considering a simplified model in which all ^{13}C labels flow from pyruvate C3 to neuronal glutamate C4, which is in exchange with astroglial glutamine C4. For this simplified model, the kinetics of ^{13}C label incorporation into glutamine C4 is described by:

$$d[^{13}\text{C-Gln C4}]/dt = V_{cyc}fe(\text{Glu C4}) - V_{cyc}fe(\text{Gln C4}) - V_{dil(Gln)}fe(\text{Gln C4}) \quad [1].$$

At isotopic steady state,

$$fe(\text{Gln C4})^{\text{steady state}} = V_{cyc}fe(\text{Glu C4})^{\text{steady state}}/(V_{cyc} + V_{dil(Gln)}) \quad [2].$$

Eq. [2] clearly shows that the entire glutamine C4 turnover curve (fe(Gln C4) *vs.* time) is sensitive to V_{cyc}, even at isotopic steady state. Fig. 1 (a) and (b) show the effect of changing V_{cyc} by ±50% (0.32 (red), 0.16 (blue) and 0.48 (black) µmol/g/min) while maintaining all other parameters unchanged. In the case of $V_{dil(Gln)}$ = 0, the end point glutamine C4 intensities converge as the isotopic steady state is approached, and where the fractional enrichments of glutamine C4 and glutamate C4 are equal regardless of V_{cyc}. Much larger changes in the simulated glutamine C4 turnover curve was clearly seen in Fig. 1(b) for $V_{dil(Gln)}$ = 0.14 µmol/g/min, indicating that the glutamine C4 enrichment curve is very sensitive to changes in V_{cyc}. The importance of $V_{dil(Gln)}$ in the sensitivity of the ^{13}C turnover curves to the value of V_{cyc} was further confirmed by the expected finding of a relatively strong positive correlation between V_{cyc} and $V_{dil(Gln)}$ using the metabolic model with inclusion of astroglial dilution in cases where no constraints were applied (Table 1, the first two rows). The corresponding Pearson's product-moment correlation coefficient for V_{cyc} ~ $V_{dil(Gln)}$ was 0.87 for σ = 0.1 µmol/g and 0.74 for σ = 0.2 µmol/g, respectively. With poor or unreported goodness-of-fit, Monte Carlo analysis *per se* is meaningless because the reliability of Monte Carlo analysis critically depends on finding the global optimum to meet the expected χ^2 statistic. Local optimization methods (BFGS and Simplex) are well-known to give false minima, thus their use should be avoided in metabolic modeling analysis where the error space is multidimensional and very complex. In contrast to an earlier analysis which did not consider astroglial dilution, used local optimization methods and did not report goodness-of-fit (2), our analysis shows that V_{cyc} can be reliably determined using [1-^{13}C]glucose infusion.

References 1. Shen et al, PNAS 96, 8235 (1999). 2. Shestov et al, J Neurosci Res 85, 3294 (2007).