Evidence for Plasma Glutamine Uptake by Brain: Implications for Metabolic Modeling of ^{13}C NMR data

Anant Bahadur Patel[1], Henk De Feyter[2], Douglas L Rothman[2], Kevin L Behar[3], and Puneet Bagga[1]

[1]NMR Microimaging and Spectroscopy, Centre for Cellular and Molecular Biology, Hyderabad, Andhra Pradesh, India, [2]Diagnostic Radiolgy, Magnetic Resonance Research Center, Yale University, New Haven, CT, United States, [3]Psychiatry, Magnetic Resonance Research Center, Yale University, New Haven, CT, United States

INTRODUCTION

^{13}C Turnover of cortical amino acids from [1-^{13}C]/[1,6-$^{13}C_2$]glucose indicates that the steady state glutamine (Gln)-C4 enrichment is ~25-30% lower than glutamate-C4[1,2]. The dilution in Gln labeling from its precursor glutamate is accommodated by including a 'diluting' flow into Gln from blood, although other sources are possible[3]. However, the source of the Gln dilution has not been established experimentally. The objective of the current study was to evaluate the potential contribution of plasma Gln to cerebral amino acid labeling and the brain Gln dilution by infusing [U-$^{13}C_5$]glutamine in mice and analysing the labeling with ^{1}H-[^{13}C]-NMR spectroscopy.

MATERIALS AND METHODS

All animal experiments were performed under protocols approved by the Institute Animal Ethics Committee. C57BL6 mice were infused with [U-$^{13}C_5$]glutamine (0.25 M) for 15, 30 and 60 min using a bolus variable rate (final rate 49 μmol/min/kg) infusion protocol used previously for ^{13}C-acetate[4]. In addition, mice were also infused with ^{13}C-Gln for 60 min at different rates to evaluate the steady glutamine labeling for different plasma Gln concentrations. Three to four mice were used for each time point and for different plasma glutamine levels. At the end of the infusion, the mouse brain was frozen *in situ* in liquid nitrogen and metabolites were extracted from frozen cortical tissue[5]. The concentration and percent ^{13}C enrichment of amino acids were measured from the ^{1}H-[^{13}C]-NMR spectrum of the cortical extract recorded at 600MHz Bruker AVANCE spectrometer[6].

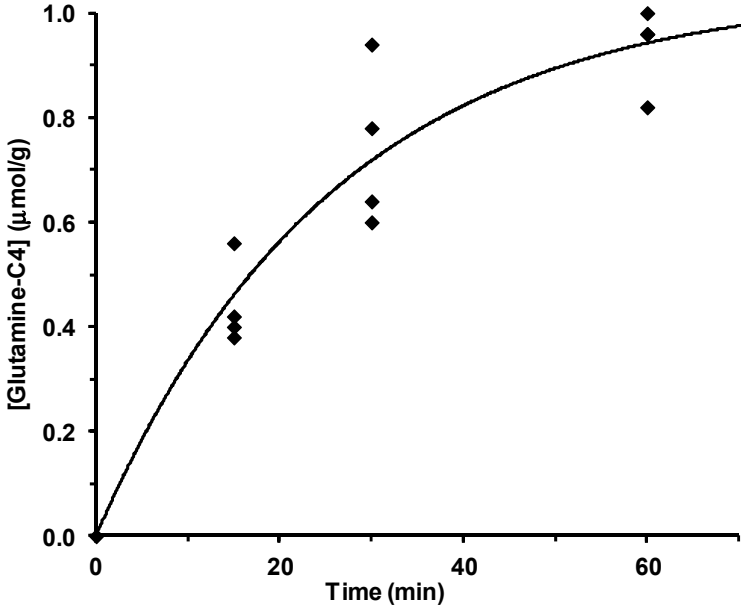

Fig. 1 ^{13}C Turnover of cortical [4-^{13}C]Gln from plasma Gln

RESULTS AND DISCUSSIONS

Intravenous infusion of [U-$^{13}C_5$]Gln led to an increase in plasma Gln level and enrichment to 2 mM and 40.0±1.0 % in less than 10 min, which remained elevated throughout the infusion. No changes were seen in brain Gln, Glu, GABA or aspartate over the range of plasma Gln levels studied (1.6-5.6 mM). ^{13}C Labeling of cortical Glu and Gln were seen above natural abundance at 15 min, and Asp and GABA labeling at 30 min or later. The steady state labeling of brain Gln_{C4} (~13-14%) from plasma Gln above ~2.4 mM appeared to be independent of plasma glutamine level over the measured range. When extrapolated to basal plasma Gln level (~1.1 mM), it could account for ~30-35% of the observed glutamine in dilution in [1-^{13}C]/[1,6-$^{13}C_2$]glucose studies (Table 1).

Table 1: Steady state ^{13}C enrichment of cortical amino acid with varying plasma glutamine

Plasma Glutamine		Cortical Amino Acid ^{13}C Enrichment (%)			Normalized ^{13}C Enrichment (%)
Concentration (mM)	Percent ^{13}C Enrichment	Glu_{C4}	$GABA_{C2}$	Gln_{C4}	Gln_{C4}
1.6±0.3	29.5±2.5	1.4±0.5	1.0±0.5	2.7±0.4	8.4±1.0
2.0±0.1	45.1±6.3	2.4±0.5	2.1±0.3	5.0±0.2	11.2±1.4
2.4±0.3	43.3±5.4	3.9±0.5	3.4±0.4	6.2±0.3	14.5±1.2
2.6±0.2	54.5±0.7	4.4±1.3	3.70.9	7.1±0.8	13.0±1.4
5.6±0.3	60.7±2.4	4.7±0.4	3.9±0.5	8.2±0.3	13.6±1.6

References: 1. Patel et al (2005) *Proc Natl Acad Sci USA* **102**:5588; 2. Chowdhury et al (2007) *J Cereb Blood Flow Metab* **27**:1895; 3. Shen et al (2009) *J Cereb Blood Flow Metab* **29**:108; 4. Patel et al (2010) *J Cereb Blood Flow Metab* **30**:1200; 5. Patel *et al* (2001) *Brain Res* **919**:207; 6. de Graaf *et al* (2003) *Magn Reson Med* **49**:37.

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