In vivo detection of dynamic ^{13}C isotopomer turnover from [U-^{13}C]-glucose

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Introduction

^{13}C isotopomers contain rich information of metabolic fluxes. Due to spectral overlap and low sensitivity, most isotopomer analysis has been performed based on *in vitro* data [e.g., 1]. With significant advancement in high field *in vivo* spectroscopy technology, *in vivo* detection of dynamic isotopomer turnover from [1,6-^{13}C]glucose has been reported recently [2]. Here we report *in vivo* detection of isotopomer patterns of high complexity and dynamic isotopomer turnover from intravenously infused [U-^{13}C]-glucose in rat brain using an INEPT-based proton-localized, polarization transfer ^{13}C MRS method and an 11.7 Tesla 89-mm bore vertical magnet.

Methods

Male adult Sprague-Dawley rats (180-200 g, n=6), which were fasted overnight before the experiment to reduce basal glucose concentration, were anaesthetized using α-chloralose throughout the experiment. Both femoral veins were cannulated for the intravenous infusion of glucose and α-chloralose, and one artery was cannulated for blood sampling to monitor blood gases and plasma glucose concentrations. All experiments were performed on a Bruker 11.7 T AVANCE spectrometer interfaced with an 89 mm i.d. vertical-bore magnet. Two concentric surface coils (^{13}C with 10.8 mm i.d./4.3 mm width, ^{1}H with 23.6 mm i.d./5.4 mm width) were used. The coils were positioned 0-1 mm posterior to bregma. Adjustment of shims was accomplished using FASTMAP/FLATNESS. Spin-echo RARE images were acquired to select an 7.5x3.75x7.5 mm^3 volume in the rat brain. *In vivo* ^{13}C NMR spectra were acquired with proton-localized, polarization transfer method [3]. Additional localization was performed on the ^{1}H magnetization using 3D-ISIS combined with outer volume suppression (OVS). Magnetization was transferred to ^{13}C using INEPT (NS=720, TR=2.5s). ^{13}C NMR spectra were collected in blocks of 30 minutes with a 5 minutes interval for re-shimming for a total acquisition of 5 hours and 10 minutes. After adjustment of NMR parameters, 99% enriched [U-^{13}C]-glucose (20 % wt/vol) was infused i.v. at 8 ml/hr over 5 minutes, followed by a continuous infusion at a lower rate which was adjusted over a small range to maintain plasma glucose concentration at 17.15±0.75 mM. Plasma samples were withdrawn every 30 minutes. Normal physiological parameters were maintained by small adjustments of respiration rate and volume (pH=7.39±0.02, pCO_2=40±3 mmHg, pO_2>100 mmHg, MABP=140-172 mm Hg).

Results and Discussion

Spectra acquired in the rat brain *in vivo* showed excellent sensitivity and spectral resolution using the INEPT technique at 11.7 T (Fig 1). The fine structure arising from ^{13}C-^{13}C J-couplings could be clearly detected in aspartate, glutamate, glutamine, and *N*-acetylaspartate. The time courses of ^{13}C label incorporation into [m-^{13}C]-glutamate, [p-^{13}C]-glutamine (m, p=2, 3,and 4), [q-^{13}C]-aspartate (q=2 and 3) and [6-^{13}C]- *N*-acetylaspartate were shown in Fig 1. As illustrated in Fig 2, at early time points, the predominant isotopomer at glutamate C4 is Glu4D45 contributed by [3-^{12}C; 4,5-^{13}C]glutamate. The contribution from [3,4,5-^{13}C]glutamate (Glu4Q) gradually dominates the isotopomer pattern at glutamate C4. Because the relative small difference between J45 and J34, the two central lines of Glu4Q coalesced after application of 10 Hz exponential line-broadening. The temporal dynamics of the isotopomer patterns of glutamine C3 and aspartate C4 is similar to glutamate C4. At later time points, the contribution from Glu3T ([2,3,4-^{13}C]glutamate) to glutamate C3 becomes dominant over that from Glu3D ([2-^{12}C; 3,4-^{13}C]glutamate + [2,3-^{13}C; 4-^{12}C]glutamate). Significant turnover of NAA C6, manifested by NAA6D at 22.52 ppm, was also observed

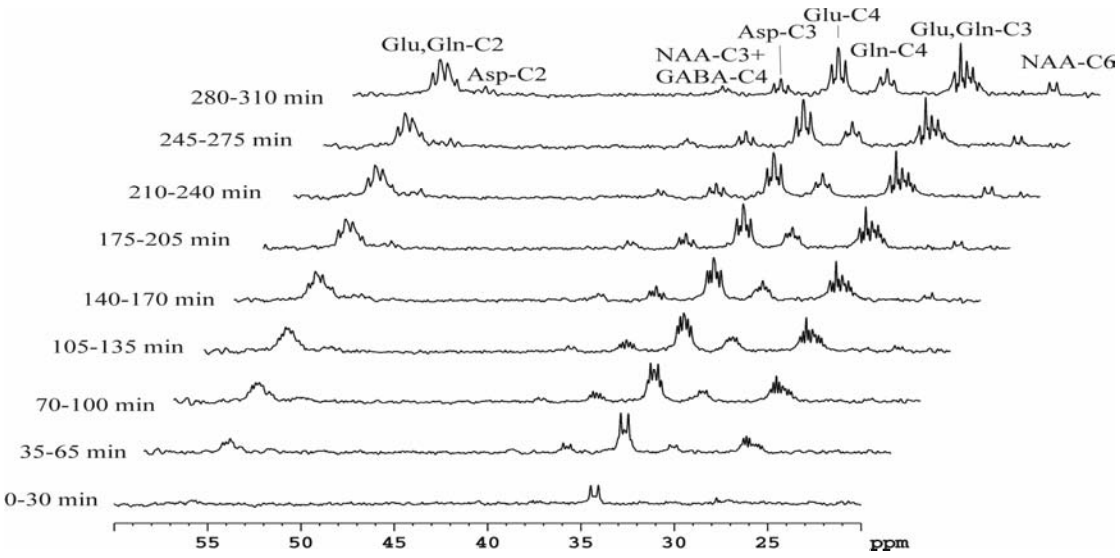

Fig. 1. Time course of combined spectra (n=6) during infusion of [U-^{13}C]-glucose. Spectra were processed using 10 Hz exponential line-broadening, zero and a minor first order phase correction without any baseline correction.

This investigation suggest that numerous isotopomers metabolized from intravenously infused [U-^{13}C]-glucose could be locally measured in the rat brain *in vivo* with a temporal resolution suitable for dynamic isotopomer analysis, which provides additional constraints for extraction of metabolic fluxes. When the carbonyl carbon of oxaloacetate is ^{13}C -labeled, regardless of the source of that label, it forms [3,4,5-^{13}C]glutamate when condensed with [1,2-^{13}C]acetylCoA. Therefore, $[Glu4Q]/[Glu\ C3]_{total}$ is a measure of the fractional enrichment (FE) of [1,2-^{13}C]acetylCoA even at nonsteady state conditions. Similarly,$[Glu4Q]/[Glu\ C4]_{total}$ is a measure of the fractional enrichment of glutamate C3 at both isotopic steady state and nonsteady state. A lag in the turnover of glutamine isotopomer from the D dominance pattern to the Q dominance pattern can be appreciated visually in Fig. 1, which is consistent with the concept of the precursor-product relationship between glutamate and glutamine and a significant cerebral glutamate-glutamine cycle.

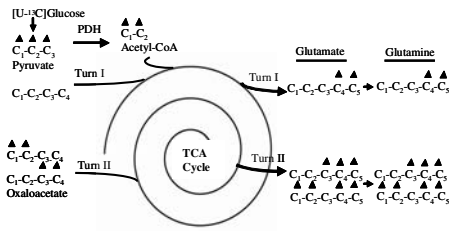

Fig. 2. Labeling patterns of glutamate and glutamine.

References

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[2] Henry *et al*, Magn Reson Med. 2003; 50:684-92.

[3] Li *et al*, ISMRM Proc. 2005:346.