

In Vivo Detection of ^{13}C Isotopomer Turnover in the Human Brain

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Introduction

Using tissue extracts and/or body fluids to measure ^{13}C isotopomers has been a valuable technique for studying metabolic pathways (1). Advances in MRS technology have also made possible in vivo detection of ^{13}C isotopomers in the aliphatic ^{13}C spectral region (2, 3). Unlike aliphatic carbons that can form complex ^{13}C isotopomer spectral patterns that may cause spectral overlap, carboxylic and amide carbons are located at the end of carbon chains. Carboxylic/amide carbons can only form singlets and doublets, resulting in significant spectral simplification. Recently, in vivo detection of ^{13}C isotopomers of rat brain in the carboxylic/amide carbon spectral region was reported using a very high magnetic field strength of 11.7 Tesla (T) (4). These rat brain studies demonstrated that it is possible to simultaneously follow the metabolism of two different substrates with one substrate ([U- ^{13}C]glucose) generating only ^{13}C doublets and the other substrate ([1- ^{13}C]acetate, [1,3- $^{13}\text{C}_2$]hydroxybutyrate, or [1- ^{13}C]ethanol) generating only ^{13}C singlets on the same carboxylic/amide carbon atom.

To investigate the feasibility of simultaneously detecting two different isotopomers in the carboxylic/amide region from the human brain at 3T, we infused [U- ^{13}C]glucose and [2- ^{13}C]glucose. [U- ^{13}C]glucose produces doublets in the carboxylic/amide carbon spectral region, while [2- ^{13}C]glucose, like [1- ^{13}C]acetate, only produces singlets in this region.

Methods

Hardware: The magnet was a standard GE 3T scanner. The proton coil was a quadrature half-volume coil. The ^{13}C coil was a single loop surface coil (inner dia.=7.5 cm) (5). A standalone proton decoupler was used to generate stochastic decoupling waveforms with bi-level outputs: low level pulsing during relaxation to generate NOE, and high level pulsing during data acquisition for proton decoupling (5). The duration of each stochastic repetition unit was 1.2 ms. Peak radio frequency power delivered into the coil was 32 W for decoupling (duty cycle=5%) and 1.0 W for NOE. Because 75% of the forward power was absorbed by the human head (mass= \sim 3.5 kg), the average SAR was \sim 0.55 W/kg.

Glucose infusion: The i.v. infusion began with [U- ^{13}C]glucose (20% w/w) for 75 minutes followed by infusion of [2- ^{13}C]glucose (20% w/w) for an additional 75 minutes. Glucose levels were kept at 130-160 mg/dL.

^{13}C MRS: Proton decoupled ^{13}C spectra were obtained from the occipital lobe of the human brain (n=2) only during the [2- ^{13}C]glucose infusion. The GE FID CSI pulse sequence was used without the phase encoding gradients (500 μs 45° hard pulse, TR=4 s, SW=5 kHz, number of data points=1024). After FASTMAP shimming, the typical water linewidth from the 125 cm³ cubical voxel was 7-8 Hz in the occipital lobe.

Results

A time course spectra of isotopomer turnover is shown in Fig 1. Each spectrum was acquired in 8.5 minutes (NS=128). Glutamate C5 originating from [U- ^{13}C]glucose appeared as a doublet, while glutamate C5 originating from [2- ^{13}C]glucose appeared as a singlet at 182.0 ppm. Decrease of the glutamate C5 doublet and increase of the glutamate C5 singlet were observed due to ending the [U- ^{13}C]glucose infusion and starting the [2- ^{13}C]glucose infusion when data acquisition began. The same time course pattern was also clearly observed for the glutamate C1 singlet and doublet at 175.4 ppm.

Fig 2 shows the sum of the spectra in Fig 1. The isotopomer pattern of glutamate C5 contained a singlet at 182.0 ppm and a doublet centered at 182.0 ppm. Similar isotopomer patterns were identified for glutamate C1 at 175.4 ppm, glutamine C5 at 178.5 ppm, aspartate C4 at 178.3 ppm, aspartate C1 at 175.0 ppm and glutamine C1 at 174.9 ppm. Lactate C1 doublet and N-acetyl-aspartate C5 doublet were also detected.

Discussion

To the best of our knowledge, this study represents the first detection of ^{13}C isotopomers in the carboxylic/amide region in human brain. Because of the lack of interference from subcutaneous lipids in the carboxylic/amide carbon region, some isotopomer patterns could be accurately measured. For example, Fig 2 shows that the ^{13}C - ^{13}C homonuclear coupling constant was 50.5 Hz for glutamate C5 (J_{45}) and 53.0 Hz for glutamate C1 (J_{12}); these values are consistent with previous J coupling values measured in rat brain at 11.7T (3, 4) and *in vitro* at 7.0T (6). Because glutamine C5 is 0.2 ppm different than aspartate C4, their isotopomer patterns were not well resolved at 3T. For the same reason, isotopomers of aspartate C1 and glutamine C1 also largely overlapped with each other. A doublet of lactate C1 was observed centered at 183.3 ppm, a finding that was not seen in previous studies that infused only [2- ^{13}C]glucose (5, 7) because [2- ^{13}C]glucose labels [2- ^{13}C]lactate only.

This study shows that it is feasible to simultaneously detect two different isotopomers in the carboxylic/amide region from the human brain at 3T. This approach may provide a new tool to simultaneously monitor ^{13}C incorporation from [U- ^{13}C]glucose and from [1- ^{13}C]acetate (a glia-specific substrate). In addition, this method may allow us to simultaneously detect the turnover from [U- ^{13}C]glucose and [2- ^{13}C]lactate or [1,3- $^{13}\text{C}_2$]hydroxybutyrate.

References

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