In Vivo Carbon-13 Spectroscopy of Rhesus Monkey Brain at 4.7T: Detecting Rapid Exchange between α-Ketoglutarate and Glutamate Using Magnetization Transfer

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INTRODUCTION

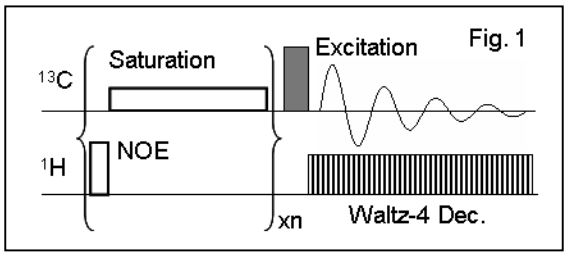
Aspartate aminotransferase (AAT) is an enzyme catalyzing the interconversion of aspartate(Asp) and α-ketoglutarate (α-KG) with oxaloacetate(OAA) and glutamate (Glu): Asp + α-KG ↔ OAA + Glu. Recently, the in vivo carbon-13 magnetization transfer (CMT) due to the AAT reaction has been discovered in the rat brain at 11.7 Tesla by saturating the carbonyl carbons (C2) of α-KG and OAA [1, 2]. In this study, the in vivo CMT effect was further validated in rhesus monkey brain at 4.7 Tesla. In particular, the AAT CMT effect was observed for the first time in the carboxylic carbon region by saturating the carboxylic carbon (C1) of α-KG.

METHOD

The experiments were performed on a Bruker spectrometer interfaced to a 4.7T 30-cm bore horizontal magnet. A ^{13}C surface coil (Dia. = 6cm) was placed inside a double-D linear ^{1}H coil formed on the surface of a 12-cm diameter cylindrical tube [3, 4]. Three female rhesus monkeys (5~6 kg) were placed in prone position and anesthetized with intravenous infusion of propofol (0.4 mg/kg/min) and intramuscular injection of dormitor (0.02 mg/kg). Administration of a solution of 20% w/w 99%-enriched [1-^{13}C]glucose began with a 10~12 ml bolus injection followed by continuous intravenous infusion to maintain glucose concentration at 20~25 mM. The pulse sequence is shown in Fig 1. This sequence was used for both C1 and C2 acquisitions. For the C2 spectra, a 1-ms adiabatic half-passage pulse (in gray) was used for the ^{13}C excitation. TR = 10000ms. Saturation of the carbonyl carbon of α-KG at 206.0 ppm was performed by applying a train of 200 ms rectangular pulses with nominal γB_{1sat} = 250 Hz interrupted by a train of hard nominal 180° proton pulses for generation of NOE. The sequence was interleaved every FID with each saturation transfer spectrum followed by a control spectrum. When control spectra were acquired, the saturation pulse was placed at an equal spectral distance from Glu C2 but on the opposite side of the α-KG C2. For the C1 spectra, a nominal 30° 50 μs hard pulse was used for ^{13}C excitation. TR = 1500 ms. The same saturation/NOE pulse train was used except that nominal γB_{1sat} = 30 Hz. Blocks of saturation and control spectra were interleaved (256 FIDs per block). For observing CMT effect in the carboxylic carbon region, the C1 carbon of α-KG at 170.3 ppm was saturated. When the control spectra were acquired, the saturation pulse was turned off. In both C1 and C2 experiments, proton decoupling was achieved by the use of the WALTZ-4 scheme with its nominal 90° pulse set to 400 μs.

RESULTS and DISCUSSION

Figure 2 shows a comparison of a saturation transfer spectrum with the corresponding control spectrum for the Glu → α-KG reaction when the carbonyl carbon of α-KG at 206.0 ppm was saturated. Spectrum 2a is the control spectrum. The corresponding saturation transfer spectrum is shown in 2b. Fig. 2c shows the difference spectrum. A total of 512 interleaved scans were accumulated. Data acquisition was initiated 90 min after the start of ^{13}C glucose infusion. 10 Hz exponential line broadening was applied before Fourier transform. A significant intensity change in Glu C2 at 55.5 ppm was observed as expected [1, 2]. The results for the carboxylic carbon region are shown in Fig. 3, where 3a is the control spectrum without any pre-irradiation and 3b is the saturation transfer spectrum. Data acquisition was initiated two hours after the start of ^{13}C-glucose infusion. 10 Hz exponential line broadening was applied before Fourier transform. A total of 8 interleaved data blocks (total NS = 2048) were accumulated. To compensate for the distortion caused by the nearby saturation pulse at 170.3 ppm, different phase and baseline corrections were made to the two spectra. An overall reduction in signal intensity in Fig. 3b was observed, which is, at least partially, due to the close proximity between the saturation pulse (170.3 ppm) and the carboxylic carbons (~175 ppm). In the control spectrum (3a), Glu C1 is higher than Gln C1. In the saturation transfer spectrum (3b), Glu C1 is lower than Gln C1 although Glu C1 at 175.3 ppm lies farther from the saturation pulse than Gln C1 at 174.8 ppm, demonstrating significant saturation transfer effect caused by the rapid exchange between α-KG and Glu although a much weaker saturation pulse (nominal γB_{1sat} = 30 Hz) was used here. Considering previous observation of relatively short ^{13}C T_1 in vivo [2, 5], the large magnetization transfer effect observed in Figs 2 and 3 indicates a very fast exchange between α-KG and Glu in the anesthetized rhesus monkey brain, consistent with our previous observation in anesthetized rat brain where the unidirectional Glu → α-KG flux has been quantified to be 78 μmol/g/min. The results shown in Figs 2 and 3, as well as those from our previous rat studies unambiguously demonstrate the existence of a fast exchange between α-KG and Glu in vivo in brain.



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