In Vivo Localized Proton Detection of Carbon-13 Magnetization Transfer Effect of Specific Enzyme Reactions

S. Xu[1], and J. Shen[1]

[1]Molecular Imaging Branch, National Institute of Mental Health, Bethesda, MD, United States

Introduction

Recently, *in vivo* ^{13}C magnetization (saturation) transfer effect of aspartate aminotransferase (AAT) and lactate dehydrogenase (LDH) reactions was discovered. The *in vivo* cerebral AAT [1] and LDH [2] flux rates have been quantified by saturating the carbonyl carbon resonances of α-ketoglutarate (α-KG), oxaloacetate or pyruvate (Pyr). While the sensitivity of the LDH reaction measurement could be dramatically increased by the use of hyperpolarized Pyr, here we developed an INEPT-based inverse ^{13}C-to-^{1}H heteronuclear polarization transfer technique for enhancing the sensitivity of ^{13}C magnetization transfer detection of enzyme kinetics *in vivo*. Inverse ^{13}C-to-^{1}H polarization transfer allows a unique combination of longer ^{13}C T_1 for maximum magnetization transfer and higher sensitivity of ^{1}H detection. In addition, the wide ^{13}C chemical shift dispersion allows minimization of RF spillover during saturation and the narrow ^{1}H chemical shift dispersion allows minimization of spatial localization error.

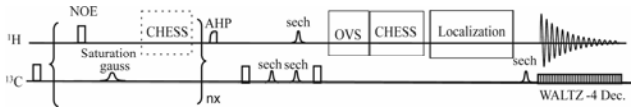

Fig. 1. Pulse sequence for localized ^{1}H detection of ^{13}C magnetization transfer of AAT and LDH reactions.

Methods

Male adult Sprague-Dawley rats (160-220 g, n = 8) were anaesthetized with isofluorane (1.5%) and infused with [1,6-$^{13}C_2$]glucose (99% enriched, 20 % wt/vol, i.v.). Plasma glucose level was rapidly raised to and maintained at 14.0 ± 2.1 mM. The AAT reaction was measured in four rats. For detecting the LDH reaction, bicuculline (1 mg/kg) was injected 15 min after the start of [1,6-$^{13}C_2$]glucose infusion (n = 4). Additional bicuculline (0.5 mg/kg per injection) was administered to maintain elevated brain lactate level during LDH measurement. All experiments were performed on a Bruker 11.7 T spectrometer interfaced to an 89 mm i.d. vertical bore magnet. Two concentric surface coils (^{13}C and ^{1}H) were used. The pulse sequence for ^{1}H detection of the ^{13}C magnetization transfer effect was shown in Fig. 1. It consists of RF saturation of α-KG C2 at 206.03 ppm or Pyr C3 at 29.08 ppm using a train of nominally 180° 2-ms Gaussian pulses and simultaneous pre-saturation of ^{1}H signals for generation of NOE enhancement and water suppression. Then glutamate (Glu) C2 at 55.20 ppm or lactate (Lac) C3 signal at 20.76 ppm was transferred to proton using an inverse ^{13}C-to-^{1}H INEPT-based polarization transfer technique. The inverse INEPT-based sequence uses adiabatic refocusing of ^{13}C spins. After generation of the heteronuclear longitudinal two-spin order, outer-volume suppression and additional water suppression were applied. Then, the longitudinal two-spin order was converted into antiphase ^{1}H transverse magnetization, localized (5 x 2.9 x 5 mm^3) and refocused before detection. During data acquisition, WALTZ-4 decoupling of ^{13}C spins was applied for a duration of 192 ms.

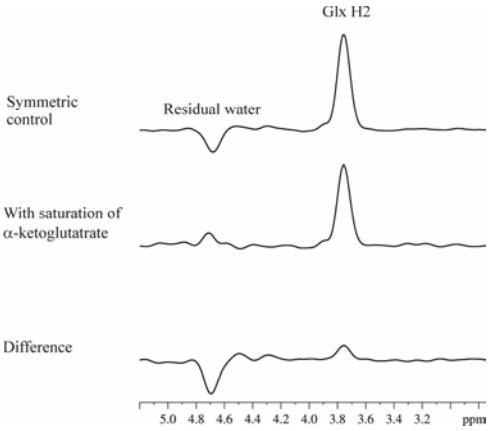

Fig. 2. Localized ^{1}H spectra (n = 4) showing the ^{13}C magnetization transfer effect due to exchange between α-KG and Glu *in vivo*.

Results

Fig. 2 shows the effect of saturating α-KG C2 on the Glu H2 resonance at 3.75 ppm. The overlapping glutamine (Gln) H2 resonance at 3.76 ppm was also present because no spectral selection of the ^{13}C spins was employed. A 16 ± 1 % change in the Glu+Gln (Glx) H2 signals was detected (n = 4). During the LDH experiment, the physiological variables were similar to previous studies [2]. Fig. 3 shows the effect of saturating Pyr C3 on Lac H3 at 1.32 ppm. The intensity change of the Lac H3 signal was found to be 16 ± 4 % (n = 4). Cancellation of the elevated alanine (Ala) signal at 1.47 ppm was incomplete in the difference spectrum due to partial saturation of the Ala C3 signal during the control experiment. Note that the rate of alanine aminotransferase reaction is too low in brain tissue to generate any detectable magnetization transfer effect *in vivo*.

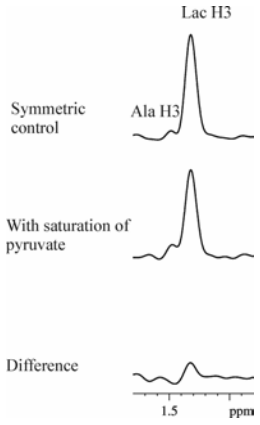

Fig. 3. Localized ^{1}H spectra (n = 4) showing the magnetization transfer effect due to exchange between Pyr and Lac *in vivo* during bicuculline-induced seizure.

Discussion

A novel ^{13}C-to-^{1}H polarization transfer technique was demonstrated here which combines ^{13}C magnetization transfer and proton observation for detecting specific enzyme reactions in the rat brain *in vivo*. Based on previously determined concentration and ^{13}C labeling of Glx [3], the signal intensity change of the Glu H2 was calculated to be 22 ± 2 % in rats corresponding to a flux rate of 72 μmol/g/min for the α-KG ↔ Glu half reaction [1], in quantitative agreement with previous determination based on direct ^{13}C detection [1]. For the LDH reaction, in addition to the Lac H3 signal at 1.32 ppm, a small Ala H3 signal at 1.47 ppm was also detected. Because the Ala C3 signal at 16.80 ppm is closer to the saturating pulse placed at 12.44 ppm during the control scans, a small negative signal of Ala was also detected in the difference spectrum which did not interfere with quantification of the change in Lac H3. In summary, the ^{13}C magnetization transfer effect of both AAT and LDH reactions was detected *in vivo* in proton spectra using the newly developed localized ^{13}C-to-^{1}H polarization transfer pulse sequence shown in Fig. 1.

References

1. Shen J. *Magn Reson Med* 2005 ; 54: 1321-1326. 2. Xu S. *et al. Magn Reson Med* in press. 3. Yang J, *et al. Neuroscience* 2005;135:927-937.