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Inverse Polarization Transfer for Detecting *in Vivo*¹³C Magnetization Transfer Effect of Specific Enzyme Reactions in ¹H Spectra

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Abstract

The wide chemical shift dispersion and long T_1 of ^{13}C have allowed determination of *in vivo* magnetization transfer effects caused by aspartate aminotransferase and lactate dehydrogenase reactions using ^{13}C MRS. In this report, we demonstrate that these effects can be observed in the proton spectra by transferring the equilibrium magnetization of ^{13}C via the one-bond scalar coupling between ^{13}C and ^1H using an inverse INEPT-based heteronuclear polarization transfer method. This inverse method allows a combination of the advantages of the long ^{13}C T_1 for maximum magnetization transfer and the high sensitivity of proton detection. The feasibility of this *in vivo* inverse polarization transfer approach was evaluated for detecting the ^{13}C magnetization transfer effect of aspartate aminotransferase and lactate dehydrogenase reactions from a 72.5 μL voxel in the rat brain at 11.7 Tesla.

Keywords

Proton Detection; Carbon-13; Magnetization Transfer Effect

Introduction

Recently, *in vivo* ^{13}C magnetization (saturation) transfer effect of aspartate aminotransferase (AAT, L-aspartate: α -ketoglutarate aminotransferase; or AST, aspartate transaminase, EC 2.6.1.1) [1], lactate dehydrogenase (LDH, EC 1.1.1.27) [2], malate dehydrogenase (MDH, EC 1.1.1.37) [3] and carbonic anhydrase (CA, EC 4.2.1.1) [4] reactions have been described. Using ^{13}C spectroscopy methods and by saturating the carbonyl carbon of α -ketoglutarate (α -KG) at 206.0 ppm in α -chloralose-anesthetized adult rat brain, the pseudo first-order rate constant of the unidirectional Glutamate (Glu) $\rightarrow$ α -KG flux was determined to be $0.13 \pm 0.01 \text{ sec}^{-1}$ (mean $\pm$ SD, $n = 11$) following intravenous infusion of [1,6- $^{13}\text{C}_2$]glucose [1]. By infusion of [2- ^{13}C]glucose and saturation of pyruvate (Pyr) C2 at 207.9 ppm the pseudo first-order rate constant of the unidirectional lactate (Lac) $\rightarrow$ Pyr flux was determined to be $0.08 \pm 0.01 \text{ sec}^{-1}$ (mean $\pm$ SD, $n = 4$) in halothane-anesthetized adult rat brain during systemic administration of GABA_A receptor antagonist bicuculline [2]. In addition, the AAT-catalyzed aspartate (Asp) $\leftrightarrow$ oxaloacetate (OAA) half reaction [1,3], the MDH-catalyzed malate +

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$\text{NAD}^+ \leftrightarrow \text{oxaloacetate} + \text{NADH} + \text{H}^+$ reaction [3], and the CA-catalyzed bicarbonate (HCO_3^-) $\leftrightarrow \text{CO}_2$ exchange reaction [4] have also been measured quantitatively *in vivo*. The LDH reaction and CA-catalyzed bicarbonate-carbon dioxide exchange have direct consequences to current ^{13}C metabolic imaging using infusion of hyperpolarized [$1\text{-}^{13}\text{C}$] pyruvate [5]. The fast exchange reactions $\text{Lac} \leftrightarrow \text{Pyr}$ catalyzed by LDH and $\text{HCO}_3^- \leftrightarrow \text{CO}_2$ by CA can obviously be measured with high spatial resolution using hyperpolarized ^{13}C imaging combined with inversion transfer [6] or exchange spectroscopy (EXSY) methods [7].

^{13}C MRS offers high spectral separation, thereby allowing clear discrimination of many metabolite resonances even at low magnetic field strength. Conventional ^{13}C MRS, which derives signals from low gyromagnetic ratio Zeeman energy difference at room temperature, suffers from inherently low sensitivity. To overcome the low sensitivity of conventional ^{13}C MRS, we used large tissue volumes for detection of the relatively small magnetization transfer effect (compared to that of the creatine kinase reaction in ^{31}P MRS) in our previous work. In the case of the CA-catalyzed bicarbonate-carbon dioxide exchange, magnetization transfer effect is expected to be undetectable if direct proton saturation and detection is employed because the labile proton of bicarbonate is not observable in proton MRS. The magnitude of magnetization transfer is expected to reduce in the cases of AAT, LDH and MDH reactions if direct proton saturation and detection is used. This is because the relatively fast T_1 relaxation processes of Glu, Asp and Lac protons which compete with the population difference caused by saturation of $\alpha\text{-KG}$, OAA, Asp or Pyr protons. The crowded proton spectrum may also make it difficult to avoid RF spillover effect unless extreme care is exercised [8–10]. These factors may explain why magnetization transfer effect of AAT, LDH and MDH reactions has escaped detection although the offset-dependent magnetization transfer effect between tissue water and metabolites has been investigated extensively using proton MRS [11,12].

Significant improvement of this situation can be achieved by combining ^{13}C magnetization transfer with high sensitivity ^1H detection. The protons bound to ^{13}C atoms can be detected by various techniques. There are three efficient ways to transfer ^{13}C equilibrium magnetization to ^1H using the one-bond J coupling between ^{13}C and its proton(s) $^1J_{\text{CH}}$: the insensitive nuclei enhanced by polarization transfer (INEPT) [13,14], the distortionless enhancement by polarization transfer (DEPT) [15] and heteronuclear Hartmann-Hahn transfer [16,17] methods. Note that techniques such as proton-observed-carbon-edited (POCE) [18], heteronuclear multiple-quantum coherence (HMQC) [19], or heteronuclear single-quantum coherence (HSQC) [20] spectroscopy methods should not be used because they detect equilibrium magnetization of ^1H instead of ^{13}C . Unlike DEPT or heteronuclear Hartmann-Hahn transfer techniques, INEPT forms intermediate longitudinal two-spin order states, which allow water and outer volume suppression (OVS) to be conveniently inserted into the sequence after creation of the longitudinal two-spin order. Here we developed an INEPT-based inverse ^{13}C -to- ^1H heteronuclear polarization transfer technique for spatially localized detection of ^{13}C magnetization transfer effect of AAT and LDH reactions *in vivo*. The long ^{13}C T_1 maximizes magnetization transfer and the wide ^{13}C chemical shift dispersion minimizes RF spillover during saturation. In the meanwhile, the high sensitivity of proton detection allows detection of the enzyme reactions in a 72.5 μL localized volume in the rat brain. The Glu+Gln (Glx) H2 methine proton at 3.75–3.76 ppm was used for detecting the ^{13}C magnetization transfer effect of the AAT reaction. Because of the close proximity between Lac H2 at 4.11 ppm and water as well as its quartet resonance structure, the more sensitive Lac H3 methyl protons was used for detection of the ^{13}C magnetization transfer effect of the LDH reaction in this study.

Materials and Methods

MR Hardware

All experiments were performed on a Bruker microimaging spectrometer (Bruker Biospin, Billerica, MA) interfaced to an 11.7 Tesla 89-mm bore vertical magnet (MagneX Scientific, Abingdon, UK). The magnet is equipped with a 57-mm inner diameter gradient (Mini0.5, Bruker Biospin, Billerica, MA, with a maximum gradient strength of 3.0 G/mm and a rise time of 100 μ s). The RF probe/animal handling system for the ^{13}C -to- ^1H heteronuclear polarization transfer spectroscopy experiments of rat brain using the vertical 89-mm bore magnet have been described recently [21]. Briefly, the inner circular loop is the ^1H coil with an inner diameter of 15 mm and a conductor width of 2.5 mm. The outer rectangular loop is the ^{13}C coil with an inner width, length and conductor width of 22.6, 25.0 and 2.5 mm, respectively. The integrated RF coils/head holder system is capable of rat head fixation (with ear pins and a bite bar), body support, physiology maintenance, coil tuning and RF shielding. The structure of RF probe/animal handling system for ^{13}C detection experiment was similar to that of the ^1H detection system except that the ^{13}C coil has an inner diameter and conductor width of 11.1 mm and 2.8 mm, respectively. The ^1H coil has an inner diameter and conductor width of 24.2 mm and 3.2 mm, respectively.

Animal Preparation

Sixteen male adult Sprague-Dawley rats (body weight: 161–223 g) were studied according to procedures approved by the National Institute of Mental Health Animal Care and Use Committee. Eight rats were used in the measurements of the ^{13}C magnetization transfer effects of ATT and LDH reactions by the INEPT-based inverse ^{13}C -to- ^1H heteronuclear polarization transfer technique (four rats for the AAT reaction, four rats for the LDH reaction). The other eight rats were used to measure the same reactions using the direct ^{13}C detection method (four rats for the AAT reaction, four rats for the LDH reaction). All rats were orally intubated and ventilated with a mixture of 70% N_2O /30% O_2 and 1.5% isoflurane. One femoral vein was cannulated for the intravenous infusion of ^{13}C -labeled glucose ([1,6- $^{13}\text{C}_2$]glucose or [1- ^{13}C]glucose) (99% enriched, Cambridge Isotope Laboratories, Inc, Andover, MA). In the case of measuring the LDH reaction, a second femoral vein was cannulated for administration of bicuculline (Sigma-Aldrich Co., St Louis, MO). One artery was cannulated for intermittent sampling of arterial blood for measurement of plasma gases and glucose concentrations using a blood analyzer (Bayer Rapidlab 860, East Walpole, MA). After surgery, anesthesia was maintained using 1.5 % isoflurane. The coils were positioned approximately 0–1 mm posterior to bregma based on separate position calibrations. Plasma glucose level was rapidly raised to and maintained at 18.3 ± 5.3 mM. For detecting the LDH reaction, bicuculline (1 mg/kg) was injected 15 min after the start of [1,6- $^{13}\text{C}_2$]glucose or [1- ^{13}C]glucose infusion. Additional bicuculline (0.5 mg/kg per injection) was administered to maintain elevated brain lactate level during data acquisition. Rectal temperature was monitored and maintained at 37.5 ± 0.5 °C using an external pump for heat exchange by water circulation (BayVotex, Modesto, CA, USA). During the AAT reaction measurement, the physiological variables were approximately maintained as following: arterial blood $\text{pO}_2 > 120$ mm Hg and pCO_2 20 – 54 mm Hg, mean blood pressure 100 – 169 mm Hg, and pH 7.10–7.36. During the LDH reaction measurement, the physiological variables were similar to our previous study using [2- ^{13}C]glucose [2]. Bicuculline treatment led to a rapid increase in mean arterial blood pressure from 122–160 mm Hg (baseline) to 155–197 mm Hg and a rapid decrease in mean heart rate from 416–478 beats per min (baseline) to 350–430 beats per min. Both mean arterial blood pressure and heart rate returned to the baseline values ~ 4 min after bicuculline administration. Blood gasses were maintained within approximately normal physiology limits ($\text{pCO}_2 = 22$ –57 mm Hg, $\text{pO}_2 > 120$ mm Hg) with few exceptions. Blood pH was found to be in the range of 6.92–7.35 due to nonrespiratory plasma acidosis caused by bicuculline-induced seizure activities [22].

Pulse Sequence Design and In Vivo MRS

Three-slice (coronal, horizontal, and sagittal) scout rapid acquisition with relaxation enhancement (RARE) images (FOV = 2.5 cm, slice thickness = 1 mm, TR/TE = 200/15 ms, rare factor = 8, 128×128 data matrix) were acquired for positioning the RF coils/head holder systems such that the gradient isocenter approximately coincides with the coil center along the z direction. The rat brain was shimmed using automatic shimming methods described earlier [23]. The pulse sequence for ^1H detection of the ^{13}C magnetization transfer effect was shown in Fig. 1. The spectroscopy voxel ($5 \times 2.9 \times 5 \text{ mm}^3$) was placed along the brain midline in the neocortex. For localized proton detection of the LDH reaction, the pulse sequence uses spectrally selective RF saturation of Pyr C3 at 29.08 ppm by a continuous train of 2 ms nominally 180° Gaussian pulses in the ^{13}C channel and a simultaneous train of nominally 180° 200 μs rectangular pulses spaced 200 ms apart in the ^1H channel for spectrally nonselective pre-saturation of thermal equilibrium ^1H signals to generate heteronuclear nuclear Overhauser enhancement (NOE) and to suppress water. The duration of the simultaneous spectrally nonselective ^1H and spectrally selective ^{13}C saturation was 9 sec. At the end of the saturation pulse trains, an optional 500 μs adiabatic half passage (AHP) pulse and crusher gradients was used for further water suppression. Then the equilibrium Lac C3 signal at 20.76 ppm was transferred to proton using an inverse, mostly adiabatic ^{13}C -to- ^1H INEPT-based polarization transfer technique. The INEPT-based inverse polarization transfer method was optimized on a phantom sample containing 10 mM [$3\text{-}^{13}\text{C}$]lactate. As shown in Fig. 1, a nonadiabatic 90°_x pulse was used for spectrally and spatially nonselective excitation of ^{13}C equilibrium signals. A pair of identical hyperbolic secant pulses (sech, 2.5 ms, $\mu = 3$, 1% truncation) were used to generate an adiabatic double spin echo with TE1 = 8.3 ms [24]. A 1.5-ms sech ($\mu = 5$, 1% truncation) was also applied to the proton channel to reintroduce heteronuclear J evolution.

After a nominal delay of $\sin^{-1}(1/2 \sqrt{3})/(\pi^1 J_{\text{CH}})$, a nonadiabatic 200 μs $90^\circ_{\pm y}$ pulse was used to convert the antiphase ($2^1\text{H}_{1z}^{13}\text{C}_y + 2^1\text{H}_{2z}^{13}\text{C}_y + 2^1\text{H}_{3z}^{13}\text{C}_y$) terms into longitudinal two-spin orders ($2^1\text{H}_{1z}^{13}\text{C}_z + 2^1\text{H}_{2z}^{13}\text{C}_z + 2^1\text{H}_{3z}^{13}\text{C}_z$). Phase cycling at the second nonadiabatic pulse facilitates cancellation of unwanted proton signals. Immediately after creation of $2^1\text{H}_z^{13}\text{C}_z + 2^1\text{H}_{2z}^{13}\text{C}_z + 2^1\text{H}_{3z}^{13}\text{C}_z$, proton outer volume suppression (OVS) using six nominal 90° sech pulses (2 ms, $\mu = 5$, 1% truncation) along the x (10 mm slab), -x (10 mm slab), y (3 mm slab), -y (5 mm slab), z (10 mm slab), -z (10 mm slab) directions plus crusher gradients, and the chemical shift-selective (CHESS) water suppression (15 ms, Gaussian 90° pulse plus gradient crushers) were executed. Then, the single-shot, fully adiabatic proton localization sequence ([25,26] and references therein) was used to convert longitudinal two-spin orders into antiphase transverse proton coherence and to achieve transverse spatial localization for selection of a $5 \text{ (x)} \times 2.9 \text{ (y)} \times 5 \text{ (z)} \text{ mm}^3$ (72.5 μL) voxel. A final hyperbolic secant pulse (2.5 ms, $\mu = 3$, 1% truncation) was applied to the ^{13}C channel to refocus heteronuclear J evolution [25,26]. After a nominal delay of $1/2^1 J_{\text{CH}}$, the in-phase transferred proton coherence is detected with simultaneous ^{13}C decoupling using WALTZ4 (9.6 ms per cycle for a total duration of 192 ms, [18]) with carrier frequency of the decoupling pulses placed at Lac C3 at 20.8 ppm. TE2 = 22.6 ms. When control spectra were acquired, the saturating pulses was placed at an equal spectral distance from the observed spin but on the opposite side of Pyr C3. The saturated and control spectra were interleaved using scheme: (control-saturated)_{NA} to minimize slow varying lactate concentration and motion artifacts. The pulse sequence used for detecting the ^{13}C magnetization transfer effect of the AAT reaction in the proton channel was similar to that described above. It was optimized using a phantom sample containing 10 mM [$2\text{-}^{13}\text{C}$] glutamate. Due to the large spectral separation between Glu C2 at 55.7 ppm and α -KG C2 at 206.0 ppm, a continuous wave pulse (CW, $\gamma B_{1\text{sat}} = 158 \text{ Hz}$) was used for spectrally selective saturation of α -KG C2. TE1 = 10.2 ms with the nominal delay used for reintroducing heteronuclear J evolution in the ^{13}C channel set to $1/4^1 J_{\text{CH}}$. The same voxel size of $5 \times 2.9 \times 5 \text{ mm}^3$ was employed. TE2 = 22.6 ms. The same WALTZ-4 decoupling sequence was used

with decoupler frequency placed at Glu C2 at 55.7 ppm. The saturated and control spectra were also interleaved as in the case of the LDH reaction.

The pulse sequences for direct ^{13}C detection of the ^{13}C magnetization transfer effect was similar to those described in our previous publications [1,2] and to the initial portion of the pulse sequence depicted in Fig. 1 for generation of heteronuclear NOE enhancement and ^{13}C saturation transfer. The methyl carbon of Pyr C3 at 29.1 ppm and the carbonyl carbon of α -KG C2 at 206.03 ppm were selectively saturated using CW pulses with a nominal $\gamma B_{1\text{sat}}$ of 79 and 158 Hz, respectively. When control spectra were acquired, the saturation pulse was placed at an equal spectral distance from the observed spin but on the opposite side of Pyr C3 or α -KG C2. The same AHP and WALTZ-4 pulses were used for excitation and subsequent proton decoupling, respectively. For determination of the Lac C3 signal in the control scan, the overlapping natural abundance background signals from subcutaneous lipids were subtracted prior to spectral quantification. All time domain spectral data were zero-filled to 16 K prior to Fourier transform.

Results

Fig. 2a shows the α -KG $\leftrightarrow$ Glu ^{13}C magnetization transfer spectra acquired using the localized ($5 \times 2.9 \times 5 \text{ mm}^3$) INEPT-based ^{13}C -to- ^1H heteronuclear polarization transfer technique and $[1,6\text{-}^{13}\text{C}_2]$ glucose infusion from one animal. Number of acquisition (NA) = 256. A total of $\text{NA} \times 2$ interleaved scans were acquired. TR = 7.1 sec. Resolution-enhancing Lorentz-Gauss transformation ($\text{lb}(\text{exponential broadening factor}) = -20$, $\text{gb}(\text{Gaussian broadening factor}) = 0.02$) was applied before Fourier transform. Only zero order phase correction was necessary. In the symmetric control spectrum and spectrum acquired with saturation of α -KG C2 at 206.03 ppm, resonances from Glu H2 (3.75 ppm), Glu H4 (2.3–2.4 ppm) and residual water (4.65 ppm) were observed. The overlapping Gln H2 resonance at 3.76 ppm was also present because of the spectrally nonselective $200 \mu\text{s } 90^\circ$ pulse employed for excitation of ^{13}C resonances. The bandwidth of the rectangular $200 \mu\text{s } 90^\circ$ ^{13}C pulse covers a range of ± 22.3 ppm at 11.7 Tesla in the ^{13}C channel (The bandwidth is defined as the width of the excitation profile at 3 dB less than the maximum amplitude). Since the spectral separation between Glu C2 and Gln C2 is only 0.5 ppm, Gln C2, although it does not carry any saturation transfer effect due to the slow rate of glutamine synthetase, was fully excited and transferred to Glx H2 (Glx H2 = Glu H2 + Gln H2). In contrast, the spectral separation between Glu C2 and Glu C4 is 21.3 ppm. Only a very small portion of Glu C4 was excited and transferred to Glu H4 as expected and shown in Fig. 2a. The more distal Gln C4 at 31.9 ppm and Glx C3 are outside of the bandwidth of the ^{13}C excitation pulse. Correspondingly, no Gln H4 (2.45 ppm) and Glx H3 (2.08–2.12 ppm) were detected in the proton spectrum shown in Fig. 2a. In Fig. 2a, the difference between the symmetric control spectrum and that with saturation of α -KG was shown in the bottom. The small Glu H4 signal was cancelled in the difference spectrum. Because α -KG C3 and C4 resonate at 31.40 and 36.66 ppm, which are far from the selectively saturated α -KG C2 resonance at 206.03 ppm, no magnetization transfer effect was expected for Glx H3 and Glx H4. The ratio of the intensity of Glu H2 in the difference spectrum to that of the Glx H2 signal in the control spectrum was determined to be $16 \pm 1 \%$ (mean $\pm$ SD, $n = 4$). Since difference between the fractional enrichment of Glu C2 and that of Gln C2 is small under our experimental condition [26], the ratio of the intensity of Glu H2 in the difference spectrum to that of the Glu H2 signal in the control spectrum can be calculated based on the known pool sizes of total Glu and Gln. Using previously determined total Glu and Gln concentration from perchloric acid extracts ($[\text{Glu}] = 10.22 \pm 0.30 \mu\text{mol/g}$ wet weight, $[\text{Gln}] = 5.17 \pm 0.31 \mu\text{mol/g}$ wet weight) [26], $(\Delta M/M^{\text{nosat}})\text{Glu}$ was calculated to be $23 \pm 2 \%$, corresponding to $k_{\text{Glu} \rightarrow \alpha\text{-KG}} = 0.12 \pm 0.01 \text{ sec}^{-1}$ for the pseudo first-order rate constant of the unidirectional $\text{Glu} \rightarrow \alpha\text{-KG}$ flux. Fig. 2b shows the same ^{13}C saturation transfer effect as shown in Fig. 2a. In Fig. 2b, the effect was measured using direct ^{13}C detection with no spatial localization other than that provided by

the ^{13}C surface transceiver coil. Infusion of $[1-^{13}\text{C}]\text{glucose}$ was used. $\text{TR} = 9.4$ sec. A total of 256×2 scans were acquired per rat. The spectra shown in Fig. 2b were summed from four rats. The difference spectrum showed significant intensity changes in Glu C2 at 55.7 ppm only. The percent change in Glu C2 signal intensity due to ^{13}C saturation transfer effect was found to be $22 \pm 1\%$ (mean $\pm$ SD, $n = 4$), corresponding to $k_{\text{Glu} \rightarrow \alpha\text{-KG}} = 0.12 \pm 0.01 \text{ sec}^{-1}$. Note that a strong resolution-enhancing window function ($\text{lb} = -30$, $\text{gb} = 0.2$) was used in Fig 2b to resolve Glu C2 at 55.7 ppm from the overlapping Gln C2 at 55.2 ppm.

The ^{13}C magnetization transfer effect due to LDH-catalyzed exchange between Pyr and Lac *in vivo* during bicuculline-induced seizure in the rat brains is as follows: Fig. 3a shows the ^1H results obtained after infusion of $[1,6-^{13}\text{C}_2]\text{glucose}$ from the localized spectroscopy voxel ($5 \times 2.9 \times 5 \text{ mm}^3$) in rat brain using the INEPT-based inverse ^{13}C -to- ^1H heteronuclear polarization transfer technique. $\text{TR} = 9.2$ sec. Total number of acquisitions was 384×2 . Resolution-enhancing Lorentz-Gauss transformation ($\text{lb} = -30$, $\text{gb} = 0.02$) was applied before Fourier transform. In the control spectrum and spectrum acquired with saturation of Pyr C3 at 29.08 ppm, both Lac H3 resonance at 1.32 ppm and alanine (Ala) H3 signal at 1.47 ppm were observed. Again, this is because the use of spectrally nonselective excitation in the ^{13}C channel, the close proximity between the resonance frequencies of Lac C3 (20.8 ppm) and Ala C3 (16.8 ppm), and elevation of Ala during bicuculline-induced seizure activities [2,22,27]. In the difference spectrum, the intensity change of the Lac H3 signal was found to be $16 \pm 4\%$ (mean $\pm$ SD, $n = 4$). The T_1 of Lac C3 was determined to be 5.6 ± 0.4 sec in a separate experiment (data not shown). The pseudo first-order rate constant for the unidirectional Lac $\rightarrow$ Pyr flux was calculated to be $0.054 \pm 0.004 \text{ sec}^{-1}$ according to procedures described in [2]. Cancellation of the elevated Ala signal at 1.47 ppm in the difference spectrum was incomplete due to partial saturation of the Ala C3 signal at 16.8 ppm during the control experiment with the irradiation frequency placed at 12.4 ppm. Note that the rate of alanine aminotransferase reaction is too low in brain tissue to generate any detectable magnetization transfer effect *in vivo* as explained earlier [2,28]. Fig. 3b showed the same ^{13}C saturation transfer effect measured using direct ^{13}C detection with no spatial localization other than that provided by the ^{13}C surface transceiver coil ($\text{TR} = 8.9$ sec, $\text{NA} = 64 \times 2$, $\text{lb} = -10$, $\text{gb} = 0.03$, $[1-^{13}\text{C}]\text{glucose}$ infusion, summed from four rats). The percent change in Lac C3 signal intensity at 20.8 ppm due to saturation of Pyr C3 at 29.1 ppm was found to be $23 \pm 4\%$ (mean $\pm$ SD, $n = 4$), corresponding to $k_{\text{Lac} \rightarrow \text{Pyr}} = 0.09 \pm 0.01 \text{ sec}^{-1}$. The relative standard deviations of the reported percent change are in agreement with the predicted values using eq. [28] given by Alger and Shulman [29].

Discussion

Although ^1H -to- ^{13}C polarization transfer methods have been extensively used for studying *in vivo* metabolism of glucose and other substrates (e.g., [30,31]), the inverse polarization transfer approach has not found *in vivo* applications until now. As *in vivo* magnetization transfer effects of more enzymes are found, which provide valuable information on specific enzymatic processes in tissue, it is imperative to evaluate the feasibility of combining ^{13}C magnetization transfer preparation and ^1H detection for characterizing these enzyme reactions *in vivo*. As shown by our results, the ^{13}C magnetization transfer effect of the AAT and LDH reactions was successfully demonstrated in the proton spectra acquired from the rat brain *in vivo* using the INEPT-based ^{13}C -to- ^1H polarization transfer technique which combines ^{13}C saturation transfer and proton detection.

The large chemical shift dispersion ($>18000 \text{ Hz}$ at 11.7 T) between the α -carbons of amino acids and the carbonyl carbons of their cognate α -keto acids provides an ideal situation for saturation transfer experiments without any significant interference from the "RF spillover" effects between the saturated spins and the observed spins. In the carbon-13 magnetization

transfer effect of rapid AAT reaction study, the chemical shift difference between α -KG C3 and Glu C3 is only 3.4 ppm. It is therefore more convenient to saturate the carbonyl carbon of α -KG at 206.03 ppm and allow magnetization (saturation) to transfer to the α -C of Glu at 55.7 ppm. The signal intensity change of the Glu H2 was calculated to be $23 \pm 2\%$ in rats using the ^{13}C -to- ^1H polarization transfer technique, in quantitative agreement with determination based on direct ^{13}C detection of the Glu C2 ($22 \pm 1\%$, Fig 2b) and with that reported previously using [1,6- $^{13}\text{C}_2$]glucose infusion and α -chloralose anesthesia [1]. Nonetheless, the presence of the overlapping Gln H2, although it carries no ^{13}C saturation transfer information and is cancelled in the difference spectra, complicates quantification by affecting the direct measurement of total Glu H2 in Fig. 2. Due to significantly increased sensitivity and the advantage of spatial localization in the proton channel, which results in much smaller localization error because of its narrow chemical shift range, inconvenience due to the presence of the inert Gln H2 resonance therefore seems to be a small price to pay.

The detection of ^{13}C magnetization transfer effect using Lac H3 is less straightforward, however. Since the signal of Lac H2 distributes among four resonance lines and it is very close to water, it is therefore impractical to use the Pyr C2 $\rightarrow$ Lac C2 $\rightarrow$ Lac H2 pathway. Instead, Pyr C3 was saturated for detection of ^{13}C magnetization transfer of the LDH reaction using the methyl protons of Lac H3 as the reporter signal. The chemical shift separation between Pyr C3 and Lac C3 is only 8.3 ppm. More importantly, Pyr C3 is also a quartet due to its one-bond scalar coupling to the three methyl protons of Pyr, making it difficult to attain spectrally selective RF saturation. The presence of Ala C3 at 16.8 ppm further complicates the situation because it was partially saturated by the control saturation pulse placed at 12.4 ppm. As Ala C3 is within the bandwidth of the 200 μs 90° ^{13}C excitation pulse centered at the frequency of Lac C3, Ala H3 was also observed in the proton spectra as shown in Fig. 3a. The $k_{\text{Lac} \rightarrow \text{Pyr}}$ determined using the inverse ^{13}C -to- ^1H polarization transfer method is significantly smaller than those determined using saturation of Pyr C2 at 207.9 ppm. This discrepancy could be due to incomplete saturation of the Pyr C3 quartet by using the train of 2-ms nominal 180° Gaussian pulses, leading to an underestimate of $k_{\text{Lac} \rightarrow \text{Pyr}}$. In addition to the Lac methyl signal at 1.32 ppm, a small Ala H3 methyl signal at 1.47 ppm was also detected. Based on our previous investigation on the LDH reaction [2], no significant signal change in Ala signal was observed in bicuculline-treated rats. Because the Ala C3 signal at 16.8 ppm is closer to the saturating pulse placed at 12.4 ppm during the control scans, a small negative signal of Ala was also detected in the difference spectrum in this study which may have also contributed to the apparent underestimation of $k_{\text{Lac} \rightarrow \text{Pyr}}$. On the other hand, the percent change in Lac C3 signal due to the magnetization transfer effect from saturating Pyr C3 was $23 \pm 4\%$ (mean $\pm$ SD) based on direct ^{13}C detection. The $k_{\text{Lac} \rightarrow \text{Pyr}}$ determined using the direct ^{13}C MRS method ($k_{\text{Lac} \rightarrow \text{Pyr}} = 0.09 \pm 0.01 \text{ sec}^{-1}$) agrees well with our previous measurement using [2- ^{13}C] glucose infusion and saturation of Pyr C2 [2]. This is explained in the following: In the direct ^{13}C MRS method, a nominal γB_1 sat of 158 Hz was used for saturation of the Pyr C3 quartet. Unlike the Gaussian pulses, the width of the frequency response of the CW pulse has a strong dependence on B_1 inhomogeneity. This dependence therefore allows more complete saturation of the Pyr C3 quartet in tissue volume closer to the coil. It should be pointed out, nonetheless, that [1,2- $^{13}\text{C}_2$]glucose infusion and a ^{13}C - ^{13}C homonuclear polarization transfer process, commonly used in multidimensional high-resolution NMR spectroscopy studies of fully ^{13}C -labeled proteins, could be employed to enable the Pyr C2 $\rightarrow$ Lac C2 $\rightarrow$ Lac C3 $\rightarrow$ Lac H3 pathway to overcome the difficulties in spectrally selective saturation of the Pyr C3 quartet as described above.

Finally, it should be noted that the α -H of Glu is also in exchange with water. This exchange, however, is very inefficient [32]. Based on previous deuterium exchange experiment [33], the rate of this proton exchange reaction is orders of magnitude slower than the unidirectional pseudo first-order rate constant $k_{\text{Glu} \rightarrow \alpha\text{-KG}}$ catalyzed by AAT. Therefore, basically no loss of

Glu H2 to water is expected due to the rapid signal detection after conversion of Glu C2 to Glu H2 using the inverse polarization transfer method. The exchange of the α -H of Lac with water is similarly slow. The methyl protons of Lac, which was used as the reporter signal of the ^{13}C magnetization transfer effect in this study, are considered to be non-labile protons. In summary, the exchange of protons of Glu and Lac with water can be safely ignored when using inverse ^{13}C - ^1H polarization transfer method for detecting ^{13}C magnetization transfer effect using proton reporter signals.

In conclusion, we have demonstrated the feasibility of detecting the ^{13}C magnetization transfer effects caused by the rapid exchanges between α -KG and Glu catalyzed by AAT and between Pyr and Lac catalyzed by LDH *in vivo* in the proton spectra using the localized INEPT-based ^{13}C -to- ^1H heteronuclear polarization transfer technique. Further extension of this strategy using inverse ^{13}C -to- ^1H DEPT or Hartmann-Hahn transfer is also feasible.

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Fig. 1.

Pulse sequence for localized ^1H detection of ^{13}C magnetization transfer effect. For clarity, depiction of the gradient pulses was omitted. Details were given in the text. Briefly, for localized proton detection of the LDH reaction, Pyr C3 was irradiated using a continuous train of 2 ms nominally 180° Gaussian pulses and a simultaneous train of nominally 180° 200 μs rectangular pulses spaced 200 ms apart. At the end of the saturation pulse trains, an optional 500 μs adiabatic half passage (AHP) pulse and crusher gradients was used for further water suppression. Then a nonadiabatic 90°_x pulse was used for excitation of ^{13}C equilibrium signals. A pair of identical hyperbolic secant pulses (sech, 2.5 ms, $\mu = 3$, 1% truncation) were used to generate an adiabatic double spin echo with an ^{13}C echo time (TE1) of 8.3 ms. A 1.5-ms sech pulse was also applied to the proton channel to reintroduce heteronuclear J evolution. A nonadiabatic 200 μs $90^\circ_{\pm y}$ pulse was used to convert the antiphase terms into longitudinal two-spin orders. Then, proton outer volume suppression using six nominal 90° sech pulses (2 ms, $\mu = 5$, 1% truncation) along the x (10 mm slab), -x (10 mm slab), y (3 mm slab), -y (5 mm slab), z (10 mm slab), -z (10 mm slab) directions plus crusher gradients, and CHESS water suppression (15 ms, Gaussian 90° pulse plus gradient crushers) were executed. Finally, the single-shot, fully adiabatic proton localization sequence was used to convert longitudinal two-spin orders into antiphase transverse proton coherence and to achieve transverse spatial localization for selection of a $5\text{ (x)} \times 2.9\text{ (y)} \times 5\text{ (z)}\text{ mm}^3$ (72.5 μL) voxel. A hyperbolic secant pulse was applied to the ^{13}C channel to refocus heteronuclear J evolution. After a nominal delay of $1/2 J_{\text{CH}}$, the in-phase transferred proton coherence is detected with simultaneous ^{13}C decoupling using WALTZ4. The ^1H echo time TE2 = 22.6 ms.

Fig. 2.

(a) The α -KG $\leftrightarrow$ Glu ^{13}C magnetization transfer spectra acquired using the localized ($5 \times 2.9 \times 5 \text{ mm}^3$) INEPT-based ^{13}C -to- ^1H heteronuclear polarization transfer technique and $[1,6\text{-}^{13}\text{C}_2]\text{glucose}$ infusion from one animal. A total of 256×2 interleaved scans were acquired. $\text{TR} = 7.1 \text{ sec}$. $\text{lb} = -20$, $\text{gb} = 0.02$. In the upper and middle traces, resonances from Glx H2 (Glu H2 + Gln H2) (3.75–3.76 ppm), Glu H4 (2.3–2.4 ppm) and residual water (4.65 ppm) were observed. Note that the small Glu H4 signal was cancelled in the difference spectrum.

(b) The same ^{13}C saturation transfer effect as shown in (a) except that the effect was measured using direct ^{13}C detection and infusion of $[1\text{-}^{13}\text{C}]\text{glucose}$. $\text{TR} = 9.4 \text{ sec}$. A total of 256×2

scans were acquired per rat. $lb = -30$, $gb = 0.2$. The spectra were summed from four rats. The difference spectrum showed significant intensity changes in Glu C2 at 55.7 ppm only.

Fig. 3.

(a) The ^1H results obtained after infusion of $[1,6-^{13}\text{C}_2]\text{glucose}$ from the localized spectroscopy voxel ($5 \times 2.9 \times 5 \text{ mm}^3$) in rat brain using the INEPT-based inverse ^{13}C -to- ^1H heteronuclear polarization transfer technique. $\text{TR} = 9.2 \text{ sec}$. $\text{lb} = -30$, $\text{gb} = 0.02$. Total number of acquisitions was 384×2 . In the control spectrum and spectrum acquired with saturation of Pyr C3 at 29.08 ppm, both Lac H3 resonance at 1.32 ppm and alanine Ala H3 signal at 1.47 ppm were observed. Cancellation of the elevated Ala signal at 1.47 ppm in the difference spectrum was incomplete due to partial saturation of the Ala C3 at 16.8 ppm signal during the control experiment with the irradiation frequency placed at 12.4 ppm. (b) The same ^{13}C saturation transfer effect measured using direct ^{13}C detection with no spatial localization other than that provided by

the ^{13}C surface transceiver coil ($\text{TR} = 8.9 \text{ sec}$, $\text{NA} = 64 \times 2$, $\text{lb} = -10$, $\text{gb} = 0.03$, $[1\text{-}^{13}\text{C}]\text{glucose}$ infusion, summed from four rats)