Localized In Vivo Hyperpolarization Transfer Sequences

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In vivo localized and fully adiabatic homonuclear and heteronuclear polarization transfer experiments were designed and performed in the rat brain at 9.4 T after infusion of hyperpolarized sodium [1,2-13C2] and sodium [1-13C] acetate. The method presented herein leads to highly enhanced in vivo detection of short-T1 13C as well as attached protons. This indirect detection scheme allows for probing additional molecular sites in hyperpolarized substrates and their metabolites and can thus lead to improved spectral resolution such as in the case of 13C-acetate metabolism. Magn Reson Med 68:349–352, 2012. ©2011 Wiley Periodicals, Inc.

Key words: hyperpolarization; dynamic nuclear polarization; 13C spectroscopy; polarization transfer; brain; metabolism

Hypermolarization methods designed to enhance the nuclear spin polarization by several orders of magnitude have recently dramatically widened the capability of MR to study biological processes in vivo (1–4). Among the available hyperpolarization schemes, dissolution dynamic nuclear polarization (DNP) is the most versatile method as it can in principle be applied to any molecule (5). Hyperpolarized 13C MR allows real-time detection of rapid metabolic processes, e.g., several enzymecatalyzed reactions have been studied in vitro and in vivo (1). However, the time delay between the DNP process and the in vitro or in vivo MR measurements as well as the finite uptake and metabolic rates of the biologically relevant organisms restrict the application of the technique to nuclear spins with long-T1. Consequently, in vitro and in vivo DNP-enhanced metabolic studies have been so far limited to the detection of nonprotonated low-γ-nuclei such as 13C-labeled carbonyls or 15N-labeled quaternary amines. However, to fully understand, control, or model the biochemical transformations taking place in complex biological systems through isotopomer analysis, it would be useful to probe several molecular sites. For instance, in the case of acetate, the long-T1 site of the substrate is the carboxyl position. Unfortunately, the biochemical transformation taking place during 13C acetate metabolism via, e.g., tricarboxylic acid (TCA) cycle does not lead to strong transformations of the chemical environment of the 13C label. As a consequence, the chemical shift of the carboxyl 13C is only slightly affected through its transfer from one metabolite to another within the TCA cycle and the following metabolic steps (6). This renders the differentiation between the substrates and its metabolic products difficult. This is particularly challenging in vivo, as the spectral resolution in tissues is more limited than in vitro. Recently, polarization transfer methods were proposed for in vitro DNP-enhanced high-resolution applications in liquid-state NMR allowing the enhancement of short-T1 nuclear spins via J-coupling-mediated transfer from the large spin polarization of the long-T1 nuclear spins in various molecules (7–9).

In this article, we present an in vivo hyperpolarization transfer sequence to enhance the signal of short-T1 nuclear spins. The proposed method allows for the in vivo localized detection of aliphatic carbons or even protons and thus offers the possibility to increase the spectral resolution or obtain additional information on the chemical environment of specific nuclear spins in biomedical hyperpolarized MR experiments. We demonstrate the feasibility and the potential of the method on hyperpolarized acetate in rodent brain.

MATERIALS AND METHODS

Carbon nuclear spins in a 4.5 M glassy frozen solution of sodium [1,2-13C2] acetate or sodium [1-13C] acetate (1:2 EIOD:D2O v/v containing 30 mM 2,2,6,6-tetramethyl-1-piperidinol) were prepared as previously described (10) and were dynamically polarized using a custom-designed DNP polarizer operating at 5 T and 1 ± 0.05 K. After 120 ± 10 min, the polarization reached 13 ± 1% and the frozen mixture was rapidly dissolved in 5 mL of superheated (185 ± 5°C) deuterated water and transferred into a plastic infusion pump located inside a 9.4 T/31 cm actively shielded animal scanner (Magnex; Refs. 11,12). A volume of 2.2 mL of hyperpolarized solution (5.2 mmol/kg) was infused within 9 s to Sprague-Dawley rats (350 g) through a catheter placed in the femoral vein. The animals were anesthetized using 1.5% isoflurane and their physiology was monitored during the experiments. All experiments were approved by the local ethics committee. Measurements were carried out on an direct drive console (Varian) using a home-built single loop 10-mm-diameter quadrature 1H surface coil with a 10-mm-diameter three loops 13C surface coil (13). In all experiments, the sequence initiation was triggered by an
external 5 V signal immediately after infusion, i.e., 12 s after dissolution. Homonuclear and heteronuclear polarization transfer schemes were designed to acquire DNP-enhanced localized spectra of short-$T_1$ nuclei in a 9.4 T using a surface coil for both excitation and detection. Two pulse sequences were implemented (Fig. 1): the first one was designed to transfer the $^{13}$C polarization to $J$-coupled $^{13}$C spins and was demonstrated following the infusion of hyperpolarized sodium $[1,2-{^{13}}$C$_2]$ acetate (Fig. 1a); the second one was designed to transfer the $^{13}$C polarization to $^1$H spins via the long distance $J$-coupling following the acquisition time to become detectable in phase $S_x$ magnetization which is largely enhanced. To compensate for the $B_1$ inhomogeneities inherent to surface coils, hyperbolic-secant adiabatic pulses were used (16). To remove all unwanted residual $^{13}$C transverse magnetization in the homonuclear sequence, homospoil gradients denoted HSG in Fig. 1a were added after the second 90$^\circ$ pulse. In the homonuclear transfer sequence, due to the strong $J_{CH} = 127$ Hz that was determined in high resolution (not shown), it is essential to apply proton decoupling to improve the resolution and the signal-to-noise ratio. In addition, the small but non-negligible losses (estimated at 5% from calculation) due to the $J_{CH} = 6$ Hz can be avoided by decoupling during the evolution delay $\Delta$ in the homonuclear sequence. To achieve suppression of the strong water signal in the heteronuclear $^1$H detected sequence, coherence-selective gradients denoted CSG in Fig. 1b were incorporated into the sequence (17).

**RESULTS**

Sequence parameters were optimized using a phantom containing sodium $[1,2-{^{13}}$C$_2]$ acetate and sodium $[1-{^{13}}$C$]$ acetate for the homonuclear and heteronuclear transfers, respectively. The sequences were then applied to acquire the methyl $^{13}$C and $^1$H signal of hyperpolarized acetate.
in vivo in the rat brain following the infusion of hyperpolarized sodium [1,2-13C2] acetate or sodium [1-13C] acetate solutions. In the homonuclear (13C-13C) case, the intrinsically large chemical shift dispersion of the 13C nuclei allowed to selectively excite the carboxyl carbon without affecting the aliphatic region (and vice versa) due to the limited radiofrequency bandwidth of the adiabatic radiofrequency pulse (3.5 kHz). 13C-13C polarization transfer was achieved via the J_CCH coupling (J_{CCH} = 52 Hz) in sodium [1,2-13C2] acetate by setting the delay \( \Delta \) between the two first 90° pulses equal to 1/2J_{CCH} (see Fig. 1a). The time domain signal (free induction decay) following the last pulse starts from the nondetectable I_xS_x coherence that is converted into the observable S_x coherence and evolves during the acquisition to become a detectable in phase term, namely S_x. The sine-like envelope of the free induction decay results in an antiphase frequency spectrum (Fig. 2).

In the heteronuclear 13C-1H experiments, the polarization transfer was performed through the weak but non-negligible \( J_{CCH} \) coupling (\( J_{CCH} = 6 \) Hz) between the carboxyl carbon and the methyl protons in sodium [1-13C] acetate. Spin multiplicity of the three equivalent methyl protons coupled to the carboxyl carbon has to be taken into account when setting the delay \( \Delta \) between the two first 90° pulses (see Fig. 1b). As in the homonuclear case, the free induction decay starts from the nondetectable two-spin antiphase coherence which develops during the acquisition to become a detectable coherence (Fig. 1c). A typical 1H magnitude spectrum is presented in Fig. 3. Note the absence of 1H water signal in the spectrum which was eliminated by the coherence-selective gradients (Fig. 1).

**DISCUSSION**

The sequences presented herein, which are compatible with the use of surface coils, are designed for in vivo metabolic studies with molecules containing short-T1 nuclear spin sites coupled to long-lived hyperpolarized nuclear spins via J-coupling. Thus, additional spectroscopic information from different chemical sites can be recorded, e.g., to assert the assignment of metabolites detected in hyperpolarized MR experiments. Furthermore, this type of detection can lead to improved spectral resolution by probing different nuclear spins of hyperpolarized precursors and their metabolites.

The transfer scheme presented above leads to unwanted but unavoidable signal losses due to the finite in vivo transverse relaxation. During the \( \Delta \) delay following the first pulse, the loss of spin coherence is governed by relaxation processes described by the carboxyl 13C \( T_2^* \) decay constant. A value of \( T_2^* = 53 \) ms was deduced from the full width at half maximum = 6 Hz of hyperpolarized sodium [1-13C] acetate localized 13C spectra measured in vivo (data not shown) using the relation \( T_2^* = \frac{1}{\gamma_1 B_{1H}} \). Taking into account that the transverse magnetization decays according to \( e^{-\Delta/T_2^*} \), an estimated 16% of the 13C polarization is lost after \( \Delta = 9.4 \) ms in the 13C-13C homonuclear case. The losses are about 50% in the heteronuclear 13C-1H case as the delay corresponding to the weak \( J_{CCH} = 6 \) Hz is set to \( \Delta = 40 \) ms. Additional losses occur during acquisition time as the evolution of the antiphase I_xS_x coherence that is converted into the observable S_x coherence is also subject to \( T_2^* \) relaxation. With a \( T_2^* \) of 22 ms deduced from the line-width of 1H spectrum (Fig. 3), the overall signal losses in the heteronuclear experiment were estimated to be about 90%, meaning that only 10% of the enhanced 13C polarization survives the polarization transfer sequence. The consequence of signal losses of magnetic field inhomogeneities could be recovered by incorporating two simultaneous 180° pulses on both 13C and 1H channels at a time \( \Delta/2 \) after the initial 13C 90° pulse as well as after the 1H 90° pulse. The loss of spin coherence would then be governed by \( T_2^* \) instead of \( T_2^* \), but the additional pulses might also lead to a degradation of the signal intensity especially if adiabatic pulses of non-negligible length are used. Finally, it is worth mentioning that coherence selective gradients refocus only half of the signal and in addition diffusion effects linked to the application of the coherence selection gradients can also lead to minor signal losses in the heteronuclear 13C-1H detection scheme.

Hyperpolarized MR experiments with two particular substrates, namely 13C-acetate (18) and 15N-choline (19) both of which having already been used for in vivo hyperpolarized studies, could benefit from the technique presented herein. For these two DNP precursors, the biochemical transformations of interest do not involve the chemical bonds directly attached to the long-T1 nuclear spins. Consequently, the observed chemical shift variations between the precursor long-T1 sites and the corresponding sites in the metabolites are likely to be small. The standard direct detection scheme may thus be limited in terms of spectral resolution and the precursors and metabolites spectra might overlap, especially at
low field. In specific cases, e.g., methyl $^{13}$C or $^1$H in 
$^{13}$C-acetate or $^1$H in $^{15}$N-choline and their respective
metabolites (7,9), larger chemical shift changes can be
detected at other chemical sites.

CONCLUSION

The pulse sequences presented in this note consist of
only three radiofrequency pulses and are thus simple to
implement for in vivo applications. The proposed
sequences are also compatible with surface coils as adia-
batic pulses can be applied. The indirect detection
schemes presented herein provides an alternative means
to detect in vivo transformations using DNP-enhanced
MR and extends the in vivo applicability of hyperpolar-
ized MR to short- $T_1$ nuclear spins, in particular protons.

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