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Hyperpolarized lithium-6 as a sensor of nanomolar contrast agents

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Abstract

Lithium is widely used in psychotherapy. The ^{6}Li isotope has a long intrinsic longitudinal relaxation time T_1 on the order of minutes, making it an ideal candidate for hyperpolarization experiments. In the present study, we demonstrated that lithium-6 can be readily hyperpolarized within 30 min, while retaining a long polarization decay time on the order of a minute. We used the intrinsically long relaxation time for the detection of 500 nM contrast agent in vitro. Hyperpolarized lithium-6 was administered to the rat and its signal retained a decay time on the order of 70 s in vivo. Localization experiments imply that the lithium signal originated from within the brain and that it was detectable up to 5 min after administration. We conclude that the detection of sub-micromolar contrast agents using hyperpolarized NMR nuclei such as ^{6}Li may provide a novel avenue for molecular imaging.

Keywords

hyperpolarization; DNP; lithium-6; relaxivity

Introduction

In clinical practice, lithium (Li) salts are used for the treatment of manic-depressive (bipolar) and depressive disorders (1,2). Once dissolved, lithium does not bind to any complexes and remains in its cationic form. It naturally exists as a mixture of two stable isotopes (3): lithium-6 (7.42%) and lithium-7 (92.58%). Because of its higher natural abundance, shorter relaxation times and higher relative sensitivity, ^{7}Li NMR has been established as a non-invasive tool to study lithium pharmacokinetics and distribution *in vivo* (4,5). Given the interest for psychiatric treatment, most studies have focused on the brain.

The isotope ^{6}Li has a long longitudinal relaxation time T_1 as well as a low gyromagnetic ratio and natural abundance, yielding a disadvantageously low sensitivity; hence virtually no *in vivo* studies using ^{6}Li NMR have to date been reported. However, the long T_1 of 170 s in

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H_2O at room temperature (6) and 1040 s in D_2O at 80 °C (7) makes it a suitable candidate for hyperpolarization experiments.

Dynamic nuclear polarization (DNP) (8) has recently found renewed interest in the field of medical NMR and MRI due to the rapid dissolution from a solid-state polarized sample in the polarizer to a room-temperature injectable solution (9). DNP has been reported to enhance the polarization (and thus signal strength) of nuclei such as by several orders of magnitude; however, once the sample is taken out of the polarizer this large polarization relaxes with the nuclei's intrinsic longitudinal relaxation time (T_1). Given the limited time that the signal amplification is available, research using dissolution DNP has been limited to nuclei with long T_1, primarily the ^{13}C nuclei in carboxyl groups (-COOH) with T_1 on the order of 20-60 s, such as pyruvate (10), acetate (11) and bicarbonate (12).

While much of the research has focused on ^{13}C and its potential for metabolic studies, we have recently proposed that hyperpolarization NMR could be used for the detection of small concentrations of contrast agents (13). Because ^{6}Li has an intrinsic T_1 that is severalfold longer than that of most ^{13}C nuclei, it could potentially provide enhanced signal much longer. This longer lasting of its signal can be combined with the principle that the relative effect of a contrast agent on a medium becomes more pronounced if the signal relaxes over a longer time. We have recently predicted that detection of contrast agent concentrations in the nanomolar range should be feasible, allowing novel avenues in molecular imaging.

Therefore, the aim of the present study was threefold: first, to establish the feasibility of dissolution DNP of ^{6}Li and to study the relaxation behavior of hyperpolarized ^{6}Li in the spectrometer. Second, to demonstrate that hyperpolarized ^{6}Li can detect contrast agent in the sub-micromolar concentration range. Third, to detect hyperpolarized ^{6}Li in the rat brain *in vivo*.

Methods

For the hyperpolarization of ^{6}Li, a 15 M 95% isotopically enriched lithium-6 chloride (Icon Isotopes, USA) in 33/67 vol% ethanol-d6/D_2O (Cambridge Isotope Laboratories, USA) solution was prepared. 300 μl (phantom) or 40 μl (*in vivo*) of this solution was frozen in liquid nitrogen as beads. 33 mM of TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy, 98% purity, Sigma-Aldrich, St. Louis, MO) was added as a polarizing agent. DNP of this sample and dissolution into 5 ml D_2O was performed in a custom-built 3.35 T polarizer, as previously described (11,14). Briefly, after microwave irradiation at 94 GHz (3.35 T) and T = 1.2 K, superheated (120 °C) high-pressure (~12 bar) D_2O was blown over the sample into a combined phase separator and infusion pump placed in the bore of the 9.4 T/31 cm spectrometer with 12 cm i.d. high-performance gradients (400 mT/m in 130 μs, Varian, Palo Alto, USA), positioned approximately 4 m from the DNP cryostat. 2.5 ml of the sample with a final ^{6}Li concentration of 900 mM (phantom) or 120 mM (*in vivo*) was then injected from the separator/infusion pump by a remote injection pump within ~9 s.

Signal acquisition typically started 18 s after dissolution. A custom-built 4-loop 8 mm diameter surface coil was used for ^{6}Li excitation and detection, with quadrature 14 mm ^{1}H coils used for localized MRI and shimming with FASTMAP (15).

Lithium-6 has the potential to detect low concentrations of contrast agents as follows: since hyperpolarized media have a non-regenerating initial longitudinal magnetization that will decay with an intrinsic decay time τ, a contrast agent will affect this magnetization with its longitudinal relaxivity r_1 (in $mM^{-1}s^{-1}$), with an observed decay time τ_{obs} expressed as $1/\tau_{obs}=1/\tau+r_1[CA]$, where [CA] is the contrast agent concentration. As a consequence, while a contrast agent may not have generated much contrast between a compartment with it and

one without it 1 s after a first excitation (and acquisition), 10 s later the difference in τ between the compartments may generate significant signal difference. More formally, if the signal S of a hyperpolarized medium at a time t after dissolution is described by $S = S_0 e^{-t/\tau}$, where S_0 is the starting signal, then the contrast $C=S_A/S_B$ between two compartments A and B with the same starting signal can be expressed as

$$C=\frac{e^{-t/\tau_{obs}}}{e^{-t/\tau}}=\frac{e^{-t\cdot[CA]\cdot r_1-t/\tau}}{e^{-t/\tau}}=e^{-t\cdot[CA]\cdot r_1}. \quad (1)$$

As a demonstration, let us take for example a contrast agent with r_1=5 $mM^{-1}s^{-1}$ at a concentration of 500 nM, and wait t = 100 s, which should be feasible given the relaxation times of ^{6}Li. The contrast generated after 100 s according to Eq. 1 would be C = 0.78, which, given a high enough signal-to-noise ratio (SNR) that should easily be discernible by NMR. Gd-DOTP had previously been shown to have a relaxivity r_1 = 11 $mM^{-1}s^{-1}$ for ^{6}Li (13), which should create a notable effect on the lithium signal decay over 100 s even when applied at concentrations below 1 μM.

To test the ability to detect such low concentrations of Gd-DOTP, a phantom consisting of a block of Ertacetal (a magnetically neutral plastic, Angst+Pfister, Switzerland) with two 1×1 cm^2 holes with a 0.5 mm thick bottom, separated by a 2 mm thick wall was used (n = 2 experiments). The RF coil was placed directly under the separation wall. Both holes were filled with 1.5 ml D_2O, while one of them was doped with 500 nM Gd-DOTP (16) (Gd(III)-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(methylenephosphonate)), Macrocyclics, TX). The signal of the two compartments was measured with 120 separately stored repetitions of a gradient-recalled sequence (3 ms 10° BIR-4 pulse, TR = 5 s), resulting after FFT in 1D projections that consisted of 32 points (TE = 1043 μs). These projections were then zero-filled to 256 points, and the integral of the signal of each compartment was calculated. Curve fitting was done from t = 30 to 300 s in OriginPro (OriginLab Corp, USA) using an exponential decay function.

In vivo experiments were performed on male Sprague-Dawley rats (~400 g, Charles River Laboratories, France). All experiments were approved by the local ethical committee. Animals were anesthetized with 1.5% isoflurane in a 30% O_2/70% N_2O mixture, and a femoral vein was catheterized for infusion of lithium. The rat was then inserted in the scanner, where physiology was monitored and kept stable: body temperature was kept at 37.3 ± 0.5 °C, while the respiration rate was maintained at 60 min^{-1} by adjustment of the isoflurane dose. Since the injected volume of 2.5 ml had an estimated lithium concentration of 120 mM, the dilution in an estimated blood volume of 30 ml resulted in a final blood concentration of LiCl in animals of approximately 10 mM; above the chronic therapeutic dose of 0.6-1.2 mM (2), but still almost an order of magnitude below the acute LD_{50} of 75 mM (calculated from (17)).

The relaxation constant τ was determined (n = 5 experiments) from the signal decay measured with a 10° BIR-4 pulse (18) applied every 3 s over 600 s. Following 5 Hz line broadening, the peak area was determined and the n^{th} excitation was corrected for the magnetization decrease due to the previous repetitive excitations by dividing by $\cos(10°)^{n-1}$. This correction did not account for in-flow into the sensitive volume, and as such provides an upper estimate of the *in vivo* relaxation time.

The ^{6}Li distribution was imaged in 4 experiments with a gradient echo sequence (2 ms sinc pulse) with an echo time of 2 ms and a repetition time of 100 ms: a total of 32 sequential images were thus acquired in 51.2 s The matrix size was 16×16 with a field of view of 6×6

cm^2 and a single slice of 1 cm thickness. Prior to FFT, images were zero-filled to 128×128 and corrected for DC offset. Three 10° BIR-4 pulse-acquire experiments preceded the imaging to estimate the relative signal strength and decay of the hyperpolarized ^{6}Li, thus the imaging started 25 s after dissolution.

In 2 experiments localized spectroscopy was performed every 60 s with outer volume suppression in 3 dimensions. A 6×8×10 mm^3 voxel was placed in the top of the rat brain, as close to the detection coil as possible. A 45° BIR-4 pulse was used for excitation, while three pairs of hyperbolic secant pulses performed the outer volume suppression. Due to the longer time between pulses, no correction for the flip angle was made in this case, resulting in the technique providing a lowest estimate of the *in vivo* relaxation time.

Results

In the polarizer, the lithium-6 chloride sample polarized with a time constant of ~450 s, while the enhancement factor at 1.2 K in the 3.35 T cryostat was ~135, equivalent to ~7 % total polarization or a ~12000-fold enhancement compared to room temperature polarization at 9.4 T. The sample was dissolved and transferred to the spectrometer in ~6 s.

We had previously proposed that ^{6}Li relaxation may be exquisitely sensitive to certain negatively charged contrast agents (13). We therefore sought to demonstrate the principle in a two-compartment phantom experiment. These experiments (n = 2) displayed the expected effect of the contrast agent (Fig. 1): the signal in the contrast agent-doped compartment decayed faster than that of the other compartment. The decay time τ_{pure} of the compartment without the contrast agent was 180 ± 12 s (and R^2=0.986), while that of the compartment containing the contrast agent was τ_{CA} = 100 ± 4 s (and R^2=0.995). From the expression for relaxivity ($1/\tau_{CA} = 1/\tau_{pure} + [CA]\cdot r_1$, see Eq. 1) with r_1 = 11 $mM^{-1}s^{-1}$ this resulted in [CA] = 440 nM, consistent with the estimated concentration of 500 nM. Conversely, taking the signal difference 100 s after normalizing the two decay curves and using Eq. 1, the calculated contrast agent concentration was 400 ± 20 nM.

In the *in vivo* pulse-acquire experiments (n = 5) the relaxation displayed an exponential decay with a decay time of τ = 72 ± 5 s (Fig. 2). The signal remained observable for over 3 min (Fig. 2a), despite repeated RF pulsing. The signal-to-noise ratio was approximately 10-fold lower than in the phantom experiments, ascribed to as both a lower polarized sample volume and a lower average final concentration.

All four ^{6}Li imaging experiments show a distribution of lithium signal with a maximum in the brain (Fig. 3). Prior to zero-filling the four high-intensity voxels were located inside the brain. Image intensity persisted through multiple images, with a τ in the same range as the pulse-acquire experiments described above.

In two studies we measured the in vivo ^{6}Li signal using localized spectroscopy to establish its presence in the brain (Fig. 4). The signal was still detected 5.5 min after dissolution, confirming a similar decay constant as the pulse-acquire studies (bottom trace in Fig. 4).

Discussion

The present study shows for the first time that lithium-6 can be readily hyperpolarized via dissolution DNP to a total polarization of 7 % in ~30 min. We furthermore demonstrated a unique sensitivity to contrast agents in the sub-micromolar range and the injection and detection of hyperpolarized ^{6}Li in the live rat brain.

The polarization decay appeared to be influenced by the presence of the TEMPO radical, which itself acts as a contrast agent. The more diluted in D_2O the sample is (and thus the lower the ^{6}Li and TEMPO concentrations), the longer its T_1: 1040 s in pure D_2O and 179 s in the two-compartment phantom (i.e. D_2O with TEMPO).

To test whether ^{6}Li can detect contrast agents in the sub-micromolar range we demonstrated the effect of 500nM Gd-DOTP. The detection limit, however, can be lowered substantially compared to what we demonstrated in this paper: assume for example that sensitivity is such that 10% signal difference due to Gd-DOTP can be detected, which is equivalent to setting C=0.9 in Eq. 1. The consequent calculation results in a detectable contrast agent concentration [CA] of 10 nM after 100s, and 5 nM after 200 s. We noted that the ^{6}Li signal was still detected 200 s after dissolution even in the presence of 500 nM GdDOTP. Designing even more powerful contrast agents are likely to push the detection limit into the picomolar range, which opens novel perspectives for molecular imaging.

Our studies, both by imaging and localized spectroscopy, suggest a localization of ^{6}Li to the brain. However, it is in principle possible that the signal remained confined to the vascular compartment. The images, however, suggest a predominantly cerebral localization of ^{6}Li, in particular considering that the maximum signal does not colocalize with major blood vessels. Although lithium uptake into the brain is considered slow, it is of interest to note that all studies measuring lithium uptake show already a sizeable fraction of their maximum lithium signal in the first measurement within minutes (5,19,20). Our present study suggests that a sufficient fraction of lithium enters the brain over the time of ^{6}Li hyperpolarization, i.e. 3-5 min. It is in principle possible to enhance the transient uptake of lithium in the brain by transiently opening the blood-brain barrier using e.g. mannitol. In a parallel manner, hyperpolarized ^{6}Li might be applied to detect cerebral tumors that disrupt the blood-brain barrier.

Conclusions

We conclude that ^{6}Li can a) be hyperpolarized, b) that it can be used to detect very low contrast agent concentrations and c) that it can be administered *in vivo* upon which it enters the brain in sufficient concentrations to be detected with localized spectroscopy.

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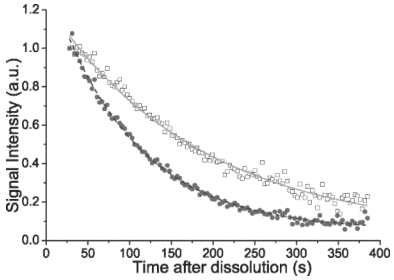

Figure 1.

Decay curves of a two-compartment phantom. Both curves were normalized to their first point. The compartment without contrast agent (upper curve) had a relaxation time of 181 ± 8 s, while the compartment with contrast agent decayed with T= 99 ± 2 s.

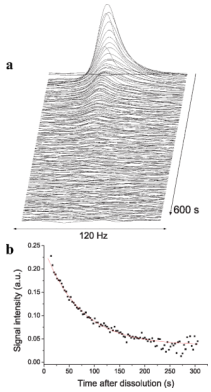

Figure 2.

a) Series of spectra of hyperpolarized lithium in the rat brain. Individual spectra were processed with 5 Hz line broadening. Signal is visible up to 300 s after dissolution. **b)** Integrals of the same peaks in the rat head, corrected for the flip angle of the excitation pulse. The solid line is an exponential fit with a decay time of 72 ± 5 s.

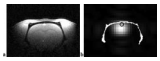

Figure 3.
a) Gradient echo axial scout image of the brain. The dorsal sagittal vein can be seen in the top of the brain as a bright spot. **b)** Lithium image with the skull from the proton image projected on top of it in white, and the dorsal sagittal vein as a black circle. The lithium signal is clearly located in the brain. Note that the distribution of the lithium signal was limited by the sensitive radius of the circular surface coil of 8 mm.

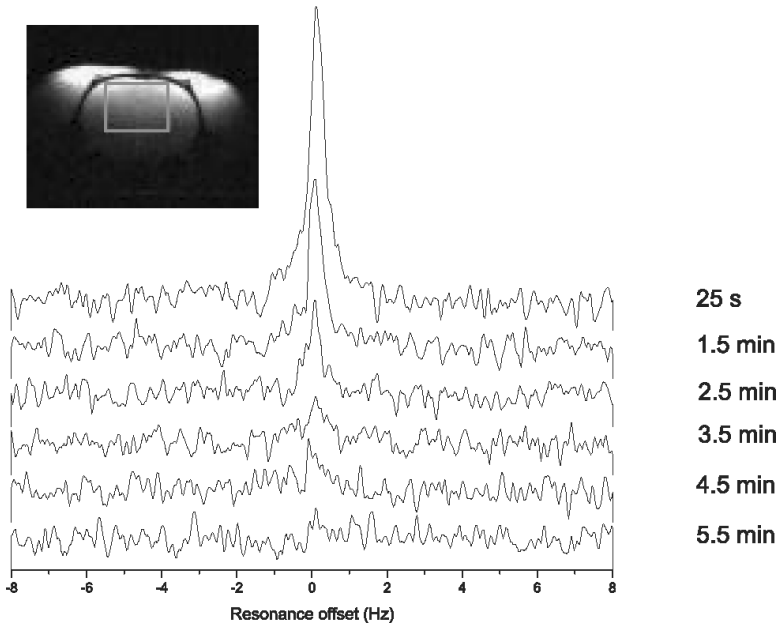

Figure 4.

Lithium-6 spectrum localized in the brain, acquired 25 s after dissolution (top trace). The spectra below were acquired every 60 s after it. The time after dissolution is mentioned to the right of each trace. After 5.5 min, a signal is still visible (bottom trace). Processing consisted of zero-filling and apodization by 5Hz prior to FFT. The location of the voxel is indicated in the inset proton image.