Available online at www.sciencedirect.com
ScienceDirect
Journal of Magnetic Resonance 186 (2007) 150–155
JMR Journal of Magnetic Resonance
www.elsevier.com/locate/jmr

Communication

Towards hyperpolarized ^{13}C-succinate imaging of brain cancer

Pratip Bhattacharya [a,b], Eduard Y. Chekmenev [a,b,c], William H. Perman [d], Kent C. Harris [a,b], Alexander P. Lin [a,c], Valerie A. Norton [b], Chou T. Tan [e], Brian D. Ross [a,b,c,*], Daniel P. Weitekamp [b]

[a] *MR Unit, Huntington Medical Research Institutes, Pasadena, CA 91105, USA*
[b] *A.A. Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, CA 91125, USA*
[c] *Rudi Schulte Research Institute, Santa Barbara, CA, USA*
[d] *Department of Radiology, St. Louis University, MO 63110, USA*
[e] *Sigma–Aldrich/Isotec, 3858, Benner Road, Miamisburg, OH 45342, USA*

Received 14 September 2006; revised 26 January 2007
Available online 30 January 2007

Abstract

We describe a novel ^{13}C enriched precursor molecule, sodium 1-^{13}C acetylenedicarboxylate, which after hydrogenation by PASADENA (Parahydrogen and Synthesis Allows Dramatically Enhanced Nuclear Alignment) under controlled experimental conditions, becomes hyperpolarized ^{13}C sodium succinate. Fast *in vivo* 3D FIESTA MR imaging demonstrated that, following carotid arterial injection, the hyperpolarized ^{13}C-succinate appeared in the head and cerebral circulation of normal and tumor-bearing rats. At this time, no *in vivo* hyperpolarized signal has been localized to normal brain or brain tumor. On the other hand, *ex vivo* samples of brain harvested from rats bearing a 9L brain tumor, 1 h or more following *in vivo* carotid injection of hyperpolarized ^{13}C sodium succinate, contained significant concentrations of the injected substrate, ^{13}C sodium succinate, together with ^{13}C maleate and succinate metabolites 1-^{13}C-glutamate, 5-^{13}C-glutamate, 1-^{13}C-glutamine and 5-^{13}C-glutamine. The ^{13}C substrates and products were below the limits of NMR detection in *ex vivo* samples of normal brain consistent with an intact blood–brain barrier. These *ex vivo* results indicate that hyperpolarized ^{13}C sodium succinate may become a useful tool for rapid *in vivo* identification of brain tumors, providing novel biomarkers in ^{13}C MR spectral-spatial images.

Keywords: PASADENA; Hyperpolarization; ^{13}C; Succinate; Acetylenedicarboxylate; 9L tumor; Glutamine; *In vivo* imaging, *Ex vivo* spectroscopy

1. Introduction

The low signal to noise ratio (SNR) in ^{13}C NMR spectroscopy is due to the fact that only a small percentage of the available ^{13}C nuclei become polarized by the externally applied magnetic field B_0 and contribute to the NMR signal in addition to low gyromagnetic ratio. Analytical chemistry applications of NMR spectroscopy overcome this low SNR problem by using concentrated samples and signal averaging. The application of NMR spectroscopy and imaging to biological systems, however, has yet to reach its full potential because of the extremely long imaging and spectroscopy acquisition times that would be required to obtain high SNR under the biological constraints of low concentration, physiological temperature, and high dielectric losses [1,2]. Nowhere is this more relevant than in the brain, where neurochemical events occur on the spatial (nm–cm) and temporal (ms–s) scales of electrical neurotransmission [3].

It is well known that metabolic substrates are transported across the blood brain barrier before undergoing neuronal and glial metabolism (Fig. 1) [4,5]. Currently *in vivo* ^{13}C MRS of human brain measures concentrations of important fuels and neurotransmitters between 1 and 10 mM

* Corresponding author. Fax: +1 626 397 5846.
E-mail address: mrs@hmri.org (B.D. Ross).

1090-7807/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmr.2007.01.017

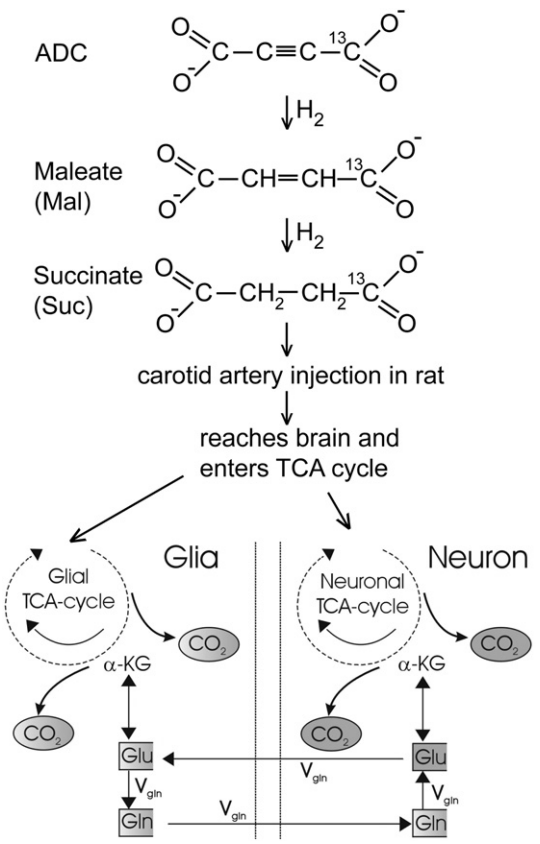

Fig. 1. A representation of intra-cerebral metabolite cycling between neurons and glia believed to underlie glutamate neurotransmission. The diagram of experimental design is shown above. Twenty-five millimolar aqueous solution of ADC precursor is hydrogenated in the polarizer to yield 3 mL of maleate and succinate products, which is then injected in the carotid artery of 9L tumor bearing rat. We propose that the hydrogenated products reach the brain through an altered blood–brain barrier in tumor tissue and enter glial and neuronal TCA cycle to yield glutamine and glutamate as final product *in vivo*.

and reaction rates of 1–5 μmol/min/g [4,5]. Two novel methods of hyperpolarization of the ^{13}C nucleus, dynamic nuclear polarization (DNP) [6,7] and parahydrogen and synthesis allow dramatically enhanced nuclear alignment (PASADENA) [8–10] provide a ^{13}C NMR signal enhancement in excess of 10,000-fold compared to Boltzmann polarization in strong magnetic field, and offer the potential for *in vivo* measurement of nanomolar quantities of metabolites and metabolic reaction rates in seconds. Several investigators have successfully hyperpolarized test reagents and imaged the resulting ^{13}C signal *in vivo* [11–16]. Subsecond Magnetic Resonance Angiography (MRA) has been demonstrated using ^{13}C reagents hyperpolarized using either PASADENA or DNP that remain in the vasculature [7,11–13,15,16]. When a hyperpolarized ^{13}C reagent exits the vasculature and enters the cells it may be metabolized, while conserving hyperpolarization of the ^{13}C nucleus, allowing the acquisition of ^{13}C images and spectra of intra-cellular metabolites. This application has been demonstrated *in vivo* by NMR detection of the conversion of ^{13}C-pyruvate to ^{13}C-lactate, ^{13}C-alanine, and ^{13}C-bicarbonate within seconds following injection of hyperpolarized ^{13}C-pyruvate at high concentration directly into mouse and dog tumors [7,13,16–18] and for skeletal and cardiac muscle of larger animals [13,16].

Although DNP ^{13}C hyperpolarization techniques have shown utility for fast *in vivo* ^{13}C NMR imaging and spectroscopy, these earlier experiments were performed at ^{13}C reagent concentrations of 300 mM, considerably higher than physiological, presenting possibly problematic biochemical and osmotic stress [10–12,19]. The purpose of this work is to demonstrate the feasibility of using physiologically relevant concentrations of PASADENA hyperpolarized molecules [19] (^{13}C-labelled succinate and maleate (Fig. 1), injected *in vivo*, as biomarkers of tumor metabolism. We observed hyperpolarized 1-^{13}C-succinate *in vivo* and its subsequent conversion to ^{13}C-glutamine and ^{13}C-glutamate in 9L brain tumor examined *ex vivo*.

2. Methods

2.1. PASADENA hyperpolarization

The instrumentation and polarization transfer technique necessary for generating hyperpolarized ^{13}C molecules is described in detail by Goldman et al. [20], Johannesson et al. [21], and Bhattacharya et al. [11]. Briefly, the parahydrogen gas is used in a chemical reaction (hydrogenation) to produce the PASADENA precursors. In order to preserve the spin correlation between the protons immediately after hydrogenation a rhodium catalyst [22,23] is used, which transfers the protons as a unit on to the precursor, without scrambling. The chemistry takes place during 4 s, at an elevated temperature (60 °C) in a reactor where a solution containing both precursor and catalyst (constituted in ultrapure water (Millipore Super-Q Plus System); final pH 7.8) is injected, into an atmosphere of 10 bar pressure of parahydrogen gas. The first step in PASADENA hyperpolarization is the molecular addition of dihydrogen, first to a catalyst which then passes the two correlated protons on to the unsaturated precursor of the target species, disodium acetylenedicarboxylate (ADC). The chemical goal is to achieve this reaction in a timescale which is small compared to spin lattice relaxation times. Relaxation losses in spin order can occur either as ^{1}H relaxation on the dihydride or relaxation of the protons or the target heteronucleus on the addition product. Longitudinal relaxation was minimized by proton irradiation in the reactor prior to polarization transfer, which traps the singlet state [24]. In order to break the symmetry to achieve PASADENA hyperpolarization on ADC, the ^{13}C label is confined to only one carbon nucleus (C1) in this symmetric molecule. The carboxyl C1 label was chosen to maximize the T_1 relaxation times for the hyperpolarized species. Theoretical analysis [20] and experimental results *in vitro* obtained with a toxic molecule hydroxyethypropionate (HEP) show ^{13}C polarization in the order of unity can be generated routinely [25]. The delays of the low field pulse

sequence [20] were chosen to be optimal for the three-spin system of maleate.

2.2. ^{13}C NMR spectroscopy of hyperpolarized samples

In vitro ^{13}C MR spectroscopy studies to confirm the chemical identity of the hydrogenated product(s) of the 1-^{13}C-ADC substrate as a result of the PASADENA hyperpolarization process were performed on a horizontal wide-bore 4.7 T MR system (Bruker Avance, Bruker, Germany). Data acquisition were started immediately after the hyperpolarized samples were placed at the center of a dual tuned $^{1}H/^{13}C$ 40 mm ID solenoid coil centered in the magnet bore. Manual transfer of the hyperpolarized materials was employed from the polarizer to the spectrometer or animal. Approximately 10 s elapsed between the completion of PASADENA hyperpolarization and the start of data acquisition in the 4.7 T MR system and 10 s on a 1.5 T GE clinical MR scanner.

2.3. In vivo ^{13}C-succinate imaging at 1.5 T

Normal rats (250–350 g wt.) were prepared for MR imaging by cannulating the right jugular vein with 120 cm long silastic tubing (0.08 mm diameter) while the rat was under anesthesia (intra-peritoneal Nembutal at 0.1 mL/100 g body weight). The procedures used on rats in this work were approved by the HMRI animal subjects committee.

MR imaging to determine the distribution of hyperpolarized ^{13}C-succinate in these normal rats was performed on a 1.5 T MR scanner (General Electric Medical Systems, Signa EchoSpeed, ESE 9.1 software, Milwaukee, WI.) The rats were sedated with intraperitoneal Nembutal (or ketamine-xylazine) as described above, placed supine on a dual tuned 12.5 cm diameter ^{1}H-^{13}C surface coil, and positioned at the center of the magnet. The manufacturer's three-dimensional FIESTA pulse sequence was modified to allow multi-nuclear 3D ^{13}C FIESTA imaging (MN-3DFIESTA) [11]. Fast MN-3DFIESTA imaging was performed before, during and after injection of ^{13}C-succinate hyperpolarized using the PASADENA technique. The MN-3DFIESTA images were acquired with TR/TE of 6.3/3.1 ms, a bandwidth of ± 62.5 kHz, sixteen 5 mm slices, 44 phase encodings over 220 mm field-of-view (FOV) and 64 readout points over 320 mm FOV to yield an isotropic spatial resolution of $5 \times 5 \times 5$ mm^3. A 4.4 M ^{13}C-acetate sphere was placed near the brain of the rat as a reference for the determination of the degree of hyperpolarization *in vivo*.

2.4. In vivo ^{13}C-succinate distribution in tumor and normal brain tissue

Brain tumors were induced in Wistar rats (approximately 250 mg) by the injection of 9L tumor cells directly into the frontal hemisphere of the brain. The rats were allowed to recover from the procedure and were imaged when tumors reached >5 mm diameter (between 10 and 15 days following tumor cell injection).

The rats were anaesthetized with ketamine-xylazine, and approximately 3 mL of hydrogenated ^{13}C-labeled product

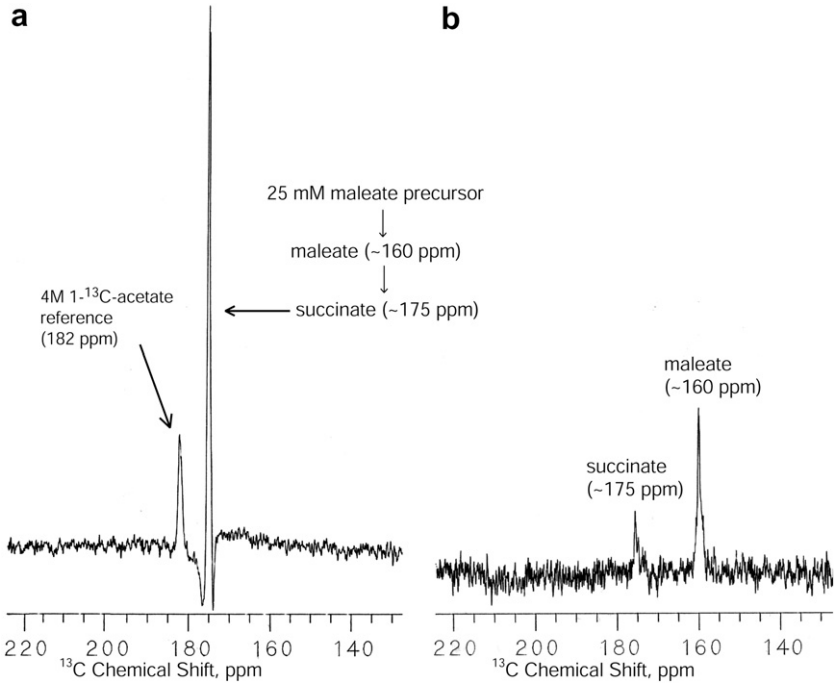

Fig. 2. (a) ^{13}C NMR spectrum of hyperpolarized 1-^{13}C-succinate from ADC precursor acquired at 4.7 T Bruker horizontal wide-bore MR system. A sphere containing 2.8 mL of 4.4 M 1-^{13}C-acetate solution is used as a reference at ~182 ppm. A single transient ^{13}C NMR spectrum revealed ~4400-fold signal enhancement with respect to Boltzmann polarization. (b) ^{13}C spectrum of the post-hyperpolarized reaction mixture at Boltzmann polarization, 128 transients.

(pH 7.8) in ultrapure water (Millipore Super-Q Plus System) was injected directly into the internal carotid artery. Following a 1 h delay to allow metabolism, the animal was sacrificed and the brain and tumor tissues were harvested and frozen in liquid nitrogen. Solid samples of brain and of brain tumor were prepared for subsequent NMR examination.

2.5. Ex vivo ^{13}C NMR spectroscopy of brain tissues

^{13}C spectroscopy of *ex vivo* samples was performed with Bruker Avance data acquisition system in 11.7 T wide bore magnet, equipped with Bruker H/X/Y VT triple resonance 4 mm magic angle spinning (MAS) probe tuned to ^{13}C resonant frequency using the X channel. Normal brain and brain tumor tissues were thawed shortly before the experiment and packed in 4 mm zirconia Bruker MAS rotors. ^{13}C MAS spectra were acquired under slow spinning conditions, low power ^{1}H decoupling and 4 °C utilizing spin echo pulse sequence and 1024 transients.

3. Results

3.1. Hydrogenation of acetylene dicarboxylate and hyperpolarization of 1-^{13}C-succinate

We chose disodium 1-^{13}C-acetylenedicarboxylate (1-^{13}C-ADC) [26] as a starting reagent for creating hyperpolarized sodium succinate using the PASADENA technique since hydrogenation of the 1-^{13}C-ADC triple bond should result in 1-^{13}C-maleate and 1-^{13}C-succinate. The ^{13}C NMR spectrum of the PASADENA reaction product of 1-^{13}C-ADC (Fig. 2a) clearly shows hyperpolarized 1-^{13}C-succinate resonating at ~175 ppm. A sphere containing 2.8 mL of 4.4 M 1-^{13}C-acetate solution with a resonance at ~182 ppm is included in the spectrum for reference. The NMR spectrum of the same PASADENA reaction solution acquired after decay of the hyperpolarized spins is shown in Fig. 2b. The expected 1-^{13}C-ADC hydrogenation products of 1-^{13}C-maleate and 1-^{13}C-succinate are both visible in this non-hyperpolarized spectrum acquired with 128 transients Fig. 2b.

3.2. Distribution of injected 1-^{13}C-succinate in rat

In vitro studies confirm that 1-^{13}C-ADC is first hydrogenated to 1-^{13}C-maleate and then further converted to 1-^{13}C-succinate in a second hydrogenation step (Fig. 1). When the resulting mixture was injected in carotid artery in normal rats, subsecond ^{13}C images indicate its delivery to vasculature and the head, but with insufficient spatial resolution at 1.5 T to distinguish brain tumor (Fig. 3) [11,27].

3.3. Metabolism of injected 1-^{13}C-succinate in rat brain

When injected via internal carotid artery into 9L tumor bearing rat, followed 1 h later by harvesting of the brain and *ex vivo* ^{13}C NMR spectroscopy, no measurable ^{13}C enriched species were observed in normal brain tissues. In contrast, 9L brain tumor tissues collected from the same rat, showed high concentrations of glutamine and glutamate enriched in C1 and C5 positions, and 1-^{13}C-maleate, in addition to the residual 1-^{13}C-succinate (Fig. 4).

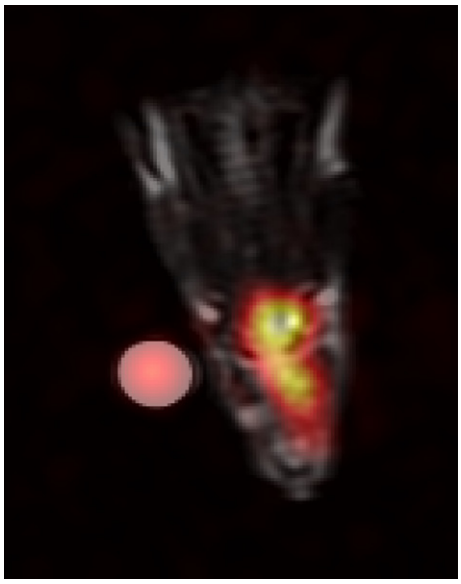

Fig. 3. *In vivo* ^{13}C 3D FIESTA imaging of rat brain with PASADENA-hyperpolarized succinate. Sub-second ^{13}C images (0.3 s) were acquired using a multi-nuclear 3D fast imaging sequence employing balanced steady-state acquisition (3D FIESTA). To allow for the short lived polarization, the 3D FIESTA ^{13}C data were acquired with a TR/TE of 6.3/3.1 ms, sixteen 5 mm slices, 44 phase encodings over 220 mm field-of-view (FOV) and 64 readout points over 320 mm FOV to yield an isotropic spatial resolution of $5 \times 5 \times 5$ mm^3. The image shown represents one slice of ^{13}C data acquired 9 s after infusion. It is overlaid on a coronal 3D fast gradient echo proton image with matching FOV and slice location acquired prior to infusion to provide anatomical correlation. All data were acquired on a 1.5 T (GE LX 9.1, Waukesha, WI) MR scanner with a dual-tuned $^{1}H/^{13}C$ custom saddle coil. The rat was anesthetized and cannulated in the carotid artery where upon 1.5 mL of 25 mM of hyperpolarized succinate was injected. For reference, a 4.4 M acetate phantom was placed next to the rat, demonstrating significant signal enhancement of hyperpolarized succinate *in vivo* given the difference in concentration (4.4 M *vs.* 0.025 M) and signal intensity.

4. Discussion and conclusion

The *in vitro* experiment shown in Fig. 2 demonstrates that ADC is a viable precursor molecule for the production of hyperpolarized 1-^{13}C-succinate using the PASADENA hyperpolarization process. The absence of a hyperpolarized 1-^{13}C-maleate peak in the spectrum shown in Fig. 2a indicates that the catalyzed hydrogenation process has proceeded past the initial hydrogenation of the triple bond which yields 1-^{13}C-maleate, to the hydrogenation of the resulting double bond which yields 1-^{13}C-succinate. Further kinetic studies and calculations are needed to establish the concentrations of maleate and succinate at the time of the polarization transfer and the optimal polarization sequence for this mixture.

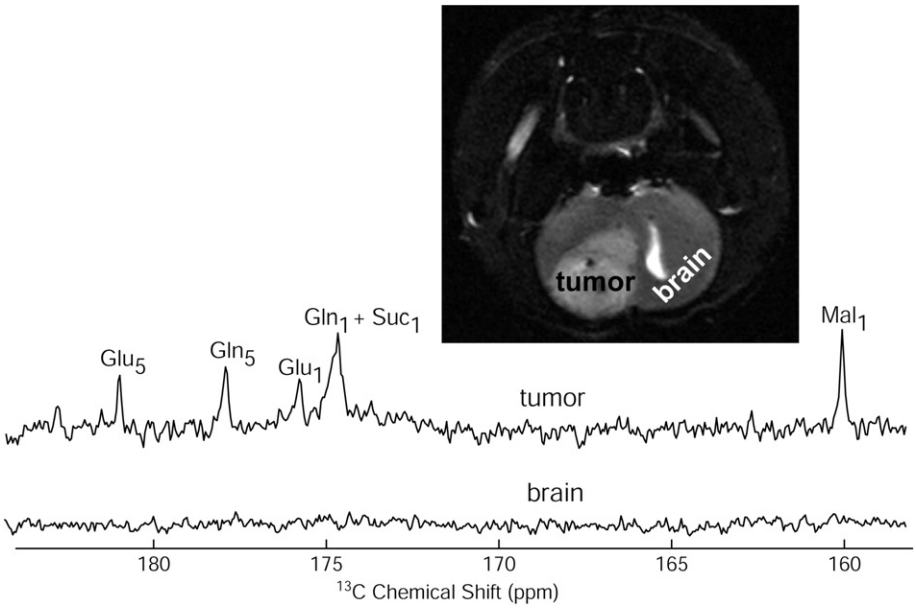

Fig. 4. *Ex vivo* MAS ^{13}C spectra of brain (lower) and brain tumor tissues (upper). 80 mg of tissue was used in each experiment. ^{13}C MAS spectra were acquired under slow spinning, 1–2 kHz conditions, low power ^{1}H decoupling and 4 °C utilizing spin echo pulse sequence with $t_{90^\circ} = 4.0$ μs, 1024 transients and recycling delay of 5 s. Representative *in vivo* RARE image of 9L tumor bearing brain is shown. ^{13}C precursors, succinate and maleate, as well as putative products of tumor metabolism, glutamine (Gln) and glutamate (Glu) are assigned based on model solutions. Note the absence of ^{13}C enrichment in normal brain tissue from the same animal.

The significant amount of ^{13}C-labeled succinate and maleate seen in the ^{13}C NMR spectrum of the tumor tissue in Fig. 4 1 h following injection of the hyperpolarized substrates indicates that 1-^{13}C-succinate and 1-^{13}C-maleate accumulate in the tumor tissue when they are not detectable in normal brain tissue. The increased concentration of 1-^{13}C-maleate, which is not a metabolic substrate for glial or neuronal metabolism, in the brain tumor spectrum is most likely due to increased accumulation due to a compromised blood–brain barrier. On the other hand, the increased concentration of 1-^{13}C-succinate in the brain tumor may be due to both increased metabolic demand and its accumulation due to the compromised blood–brain barrier. The presence of ^{13}C-labeled glutamate and glutamine, metabolic products of 1-^{13}C-succinate may act as specific tumor biomarkers. Recent studies link abnormalities in tumor TCA-cycle enzymes succinic dehydrogenase and fumarate hydratase to known oncogenes [28–30]. Subsecond ^{13}C imaging and spectroscopy performed after administration of hyperpolarized 1-^{13}C-succinate may be uniquely sensitive for the *in vivo* detection of tumors.

These experiments also indicate that it should be possible to perform *in vivo* dynamic MR imaging and spectroscopy of tumor metabolism following injection of millimolar concentrations of hyperpolarized 1-^{13}C-succinate, correctly determining *in vivo* metabolic flux of the hyperpolarized tracer administered in low (physiologic) concentrations and pH buffered solutions. The PASADENA method can produce hyperpolarized product every 2 min [11] which allows repeated *in vivo* studies of metabolism in the same animal. Furthermore, the relatively long T_1 of the carbonyl carbons and the degradation of the blood–brain barrier in tumors suggest that these species may discriminate between normal brain and metabolically active brain tumor tissues on the time scale over which hyperpolarization is maintained. We are currently investigating the ability of fast spectral-spatial imaging of hyperpolarized 1-^{13}C-succinate to capture this information from brain tumors *in vivo*. In the current chemical design of the hydrogenation experiment, T_1 of 1-^{13}C-succinate is only 6 s. Moreover, the hydrogenation reaction of 1-^{13}C-ADC is incompletely controlled, resulting in production of both maleate and succinate species in solution. Because maleate is toxic at concentrations above 10 mM [31], we would like to avoid this intermediate. Accordingly, alternative reagents with double bonds for controlled hydrogenation and proper isotope enrichment are being designed to alleviate the above limitations and permit routine production of hyperpolarized succinate.

The ability to increase the NMR signal of ^{13}C-labeled biologically relevant metabolic substrates by several orders of magnitude in the PASADENA hyperpolarization process promises to provide new methods for measuring and understanding tumor metabolism *in vivo*.

Acknowledgments

This work was generously supported by Rudi Schulte Research Institutes (E.Y.C., A.P.L.), James G. Boswell Fellowship (P.B.), Mildred Swanson/American Brain Tumor Association Fellowship (P.B.), American Heart Association Fellowship (P.B.), NARSAD Young Investigator

Award (K.C.H.), Beckmann Institute Resource Center Pilot Award (V.A.N., D.P.W.) and performed under research Grant 1R21 CA118509 from National Cancer Institute (P.B.). The polarizer was provided under loan agreement between HMRI and GE Healthcare established by Dr. Klaes Golman, Ms. Marivi Mendizabal and Jonathan Murray. Preliminary advice from Dr. Oskar Axelson and colleagues at Amersham Bioscience, Malmo, Sweden is much appreciated. We thank Dr. Keiko Kanamori (HMRI) for advice and Jan Hövener for technical support. Prof. Brian D. Ross, University of Michigan, Ann Arbor, prepared and supplied 9L tumor bearing rats.

References

[1] R. Edelman, J. Hesselink, M. Zlatkin, Clinical Magnetic Resonance Imaging, W.B. Saunders and Co., Philadelphia, 1996.
[2] Magnetic Resonance at Solid State Physics and Magnetic Resonance Service in Saclay, Bulletin D Informations Scientifiques Et Techniques (1969) 3.
[3] Modern Techniques in Neuroscience Research, Springer–Verlag, 1999.
[4] S. Cerdan, NMR in Biomedicine, Wiley, NY, 2003.
[5] B. Ross, A. Lin, K. Harris, P. Bhattacharya, B. Schweinsburg, Clinical experience with C-13 MRS in vivo, NMR Biomed. 16 (2003) 358–369.
[6] J.H. Ardenkjaer-Larsen, B. Fridlund, A. Gram, G. Hansson, L. Hansson, M.H. Lerche, R. Servin, M. Thaning, K. Golman, Increase in signal-to-noise ratio of >10,000 times in liquid-state NMR, Proc. Natl. Acad. Sci. USA 100 (2003) 10158–10163.
[7] K. Golman, J.H. Ardenkjaer-Larsen, J.S. Petersson, S. Mansson, I. Leunbach, Molecular imaging with endogenous substances, Proc. Natl. Acad. Sci. 100 (2003) 10435–10439.
[8] C.R. Bowers, D.P. Weitekamp, Transformation of symmetrization order to nuclear-spin magnetization by chemical-reaction and nuclear-magnetic-resonance, Phys. Rev. Lett. 57 (1986) 2645–2648.
[9] C.R. Bowers, D.P. Weitekamp, Para-hydrogen and synthesis allow dramatically enhanced nuclear alignment, J. Am. Chem. Soc. 109 (1987) 5541–5542.
[10] K. Golman, O. Axelsson, H. Johannesson, S. Mansson, C. Olofsson, J.S. Petersson, Parahydrogen-induced polarization in imaging: Subsecond C-13 angiography, Magn. Reson. Med. 46 (2001) 1–5.
[11] P. Bhattacharya, K. Harris, A.P. Lin, M. Mansson, V.A. Norton, W.H. Perman, D.P. Weitekamp, B.D. Ross, Ultra-fast three dimensional imaging of hyperpolarized C-13 in vivo, Magn. Reson. Mater. Phys., Biol. Med. 18 (2005) 245–256.
[12] K. Golman, R. in 't Zandt, M. Thaning, Real-time metabolic imaging, Proc. Natl. Acad. Sci. 103 (2006) 11270–11275.
[13] K. Golman, J.S. Petersson, Metabolic imaging and other applications of hyperpolarized ^{13}Cl, Acad. Radiol. 13 (2006) 932–942.
[14] K. Golman, L.E. Olsson, O. Axelsson, S. Mansson, M. Karlsson, J.S. Petersson, Molecular imaging using hyperpolarized ^{13}C, Br. J. Radiol. 76 (Spec No. 2) (2003) S118–S127.
[15] S. Mansson, E. Johansson, P. Magnusson, C.M. Chai, G. Hansson, J.S. Petersson, F. Stahlberg, K. Golman, ^{13}C imaging—a new diagnostic platform, Eur. Radiol. (2005).
[16] L.E. Olsson, C. Chai, O. Axelsson, M. Karlsson, K. Golman, J.S. Petersson, MR coronary angiography in pigs with intraarterial injections of a hyperpolarized ^{13}C substance, Magn. Reson. Med. 55 (2006) 731–737.
[17] A.P. Chen, M. Albers, S.J. Kohler, Y.-F. Yen, R.E. Hurd, J. Tropp, P. Pels, M.L. Zierhut, R. Bok, S.J. Nelson, J. Kurhanewicz, D.B. Vigneron, High-resolution hyperpolarized C-13 spectroscopic imaging of the TRAMP mouse at 3T, Proc. Int. Soc. Magn. Reson. Med. 14 (2006) 128.
[18] S.J. Kohler, Y. Yen, J. Wolber, R. in 't Zandt, A. Gram, F. Ellner, M. Thaning, A. Chen, M. Albers, R. Bok, J. Tropp, S. Nelson, D. Vigneron, J. Kurhanewicz, R. Hurd, Carbon-13 metabolic imaging at 3 T using hyperpolarized 13C-1-pyruvate, Proc. Int. Soc. Magn. Reson. Med. 14 (2006) 128.
[19] B.D. Ross, A.P. Lin, P. Bhattacharya, K.C. Harris, K. Kanamori, A. Freundlich, T.T. Tran, Limits of detection of hyperpolarized 13C in vitro and in vivo, Proc. Int. Soc. Magn. Reson. Med. 14 (2006) 128.
[20] M. Goldman, H. Johannesson, O. Axelsson, M. Karlsson, Design and implementation of C-13 hyperpolarization from para-hydrogen, for new MRI contrast agents, CR Chimie 9 (2006) 357–363.
[21] H. Johannesson, O. Axelsson, M. Karlsson, Transfer of para-hydrogen spin order into polarization by diabatic field cycling, CR Physique 5 (2004) 315–324.
[22] A. Lei, J.P. Waldkirch, M. He, X. Zhang, Highly enantioselective cycloisomerization of enynes catalyzed by rhodium for the preparation of functionalized lactams, Angew. Chem. 114 (2002) 4708–4711.
[23] T. Imamoto, New P-chirogenic diphosphines and their use in catalytic asymmetric reactions, Pure Appl. Chem. 73 (2001) 373–376.
[24] M. Carravetta, M.H. Levitt, Long-lived nuclear spin states in high-field solution NMR, J. Am. Chem. Soc. 126 (2004) 6228–6229.
[25] V.A. Norton, P. Bhattacharya, B.D. Ross, D.P. Weitekamp, New Techniques for Interconvertion of Non-Equilibrium Spin Order, 47th ENC Conference, 2006.
[26] P. Bhattacharya, B.D. Ross, Hyperpolarized imaging agents and methods of use thereof, in: U. Patent, (Ed.), U.S. Patent Office: 40127-32 Patent pending, 2005.
[27] P. Bhattacharya, V.A. Norton, K.C. Harris, A.P. Lin, W.H. Perman, B.D. Ross, D.P. Weitekamp, ^{13}C PASADENA imaging in vivo, 47th ENC Conference, 2006.
[28] M.A. Esteban, P.H. Maxwell, HIF, a missing link between metabolism and cancer, Nat. Med. 11 (2005) 1047–1048.
[29] P. Rustin, Mitochondria, from cell death to proliferation, Nat. Genet. 30 (2002) 352–353.
[30] P. Rustin, A. Munnich, A. Rotig, Succinate dehydrogenase and human diseases: new insights into a well-known enzyme, Eur. J. Hum. Genet. 10 (2002) 289–291.
[31] A. Freundlich, P. Bhattacharya, G. Techy, B. Ward, M. Ingram, B.D. Ross, Rapid toxicity screening of novel PASADENA MRI contrast agents, Proc. Int. Soc. Magn. Reson. Med. (2006) 665.