Localized Detection of Hyperpolarized [1-^{13}C]Pyruvate and its Metabolic Products in Rat Brain

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Introduction: ^{13}C NMR spectroscopy is hampered by its relatively low sensitivity and natural abundance. One way to enhance the sensitivity of ^{13}C is to use dynamic nuclear polarization (DNP) technique as previously reported [1]. Detection of [1-^{13}C]pyruvate and its metabolic products have been reported in kidney, liver and muscle [2,3]. The aim of this study was to demonstrate the feasibility of measuring ^{13}C signals of hyperpolarized ^{13}C metabolic products in the rat brain *in vivo* following injection of hyperpolarized [1-^{13}C]pyruvate.

Methods: A mixture of [1-^{13}C]pyruvic acid and OX63 trityl radical was hyperpolarized by DNP (Hypersense, Oxford Instruments, UK) for 90 min in a magnetic field of 3.35 T at approximately 1.4 K [4]. The sample was then dissolved in EDTA/water mixture to produce 4 ml of hyperpolarized solution at a concentration of ~35 mM. Experiments were performed on a 9.4T/31cm bore magnet interfaced to a Varian INOVA spectrometer. Fasted male Sprague-Dawley rats (n = 5) were injected intravenously with approximately 2.2 mL of hyperpolarized [1-^{13}C]pyruvate under isoflurane anesthesia. Injection started about 20 s after dissolution and lasted for ~6 s. *In vivo* decoupled-^{13}C NMR spectra were acquired from the rat brain using pulse-acquire with a small flip angle (4.5° at the coil center). A LASER sequence adapted for ^{13}C spectroscopy [5] was also used to acquire localized ^{13}C signals from the brain (VOI of 400 µl). Physiological condition of animals was monitored throughout the study.

Results: An *in vivo* ^{13}C-LASER NMR spectrum acquired in rat brain (Fig. 1A) 9 s after the start of injection of hyperpolarized [1-^{13}C]pyruvate showed resonances corresponding to pyruvate-C1 (172.08 ppm), pyruvate hydrate-C1 (180.42 ppm), lactate-C1 (184.3 ppm) as well as alanine-C1. Excellent localization was achieved as judged from the fact that no signal from formic acid (reference sphere placed at ^{13}C coil center) was observed in the LASER spectrum compared to the pulse-acquire spectrum (Fig 1B).
The *in vivo* time courses of ^{13}C hyperpolarized metabolites (unlocalized) were measured with a temporal resolution of 3 s (Fig. 2). The infused substrate peaked at 9 s after the infusion started and the signal gradually decreased within 60 s. Formation of hyperpolarized [1-^{13}C]lactate was also observed. The signal enhancement factor for [1-^{13}C]pyruvate was ~4000 at the time of measurement as determined on phantom, and its T_1 relaxation time was about 25 s in phantom and 20 s *in vivo*.

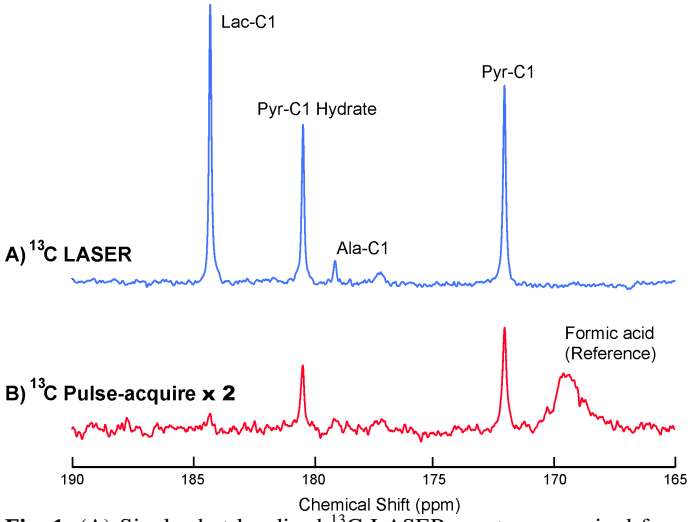

Fig. 1: (A) Single-shot localized ^{13}C-LASER spectrum acquired from the rat brain 9 s after injection of hyperpolarized [1-^{13}C]pyruvate with 90° excitation pulse and (B) ^{13}C pulse-acquire spectrum (scaled up 2 times) acquired 6 s after injection with 4.5° nominal pulse angle.

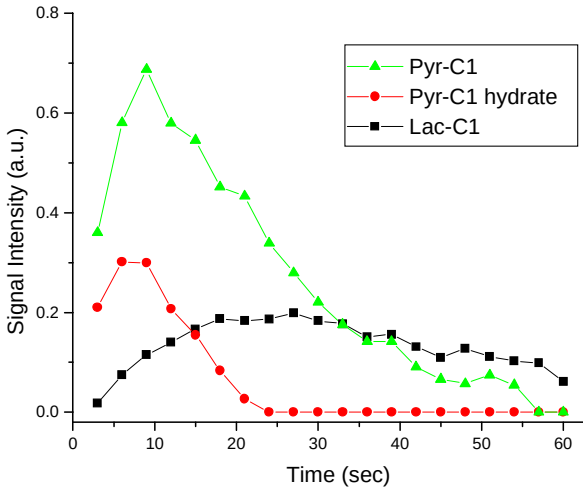

Fig. 2: Measured *in vivo* time courses of pyruvate C1, pyruvate hydrate C1 and lactate C1 after injection of hyperpolarized [1-^{13}C]pyruvate (pulse-acquire, 4.5° pulse angle, TR 3 s).

Discussion and Conclusion: The localized LASER spectrum demonstrates that a large fraction of the detected ^{13}C NMR signal comes from within the brain. In addition, the fact that the ratio pyruvate:lactate is much higher in the unlocalized spectrum than in the localized spectrum suggests that a large part of [1-^{13}C]lactate comes from brain tissue and not from blood. If both lactate and pyruvate signals were dominated by contribution from the blood, localized and unlocalized measurements would give similar ratios of pyruvate:lactate, irrespective of localization. Therefore, although LASER does not allow measurement of time courses due to the 90° excitation pulse, it gives valuable insights into the origin of NMR signals. Development of localized pulse sequences with small excitation flip angle will permit detection of localized time courses in the future. We conclude that hyperpolarized [1-^{13}C]pyruvate and its metabolic products can be measured in the rat brain *in vivo*.

References: [1] Abragam et al. Goldman Rep Prog Phys 1978; [2] Kohler et al. MRM 2007 ; [3] Golman et al. PNAS 2006 ; [4] Wolber et al. Nucl Instru Methods Phys Rev A 2004; [5] Garwood et al. JMR 2001.

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