First studies with hyperpolarized [2-^{13}C]pyruvate in the rat brain

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Introduction. Carbon-13 NMR studies with hyperpolarized molecules may result in up to 10,000-fold increase in sensitivity (1), potentially allowing the detection of metabolic events in real-time *in vivo*. Several studies have been reported with [1-^{13}C]pyruvate (2,3), but to our knowledge, there has been no report about hyperpolarized [2-^{13}C]pyruvate. In this work, we hyperpolarized [2-^{13}C]pyruvate and measured the resulting ^{13}C signals from labeled metabolites in the rat brain *in vivo*.

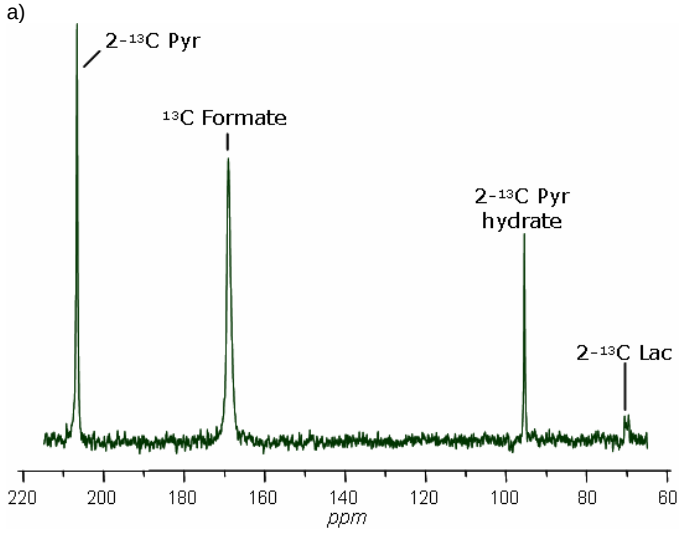
Materials and Methods. Three fasted male Sprague-Dawley rats were anesthetized with isoflurane and surgically prepared for intravenous injection of hyperpolarized [2-^{13}C]pyruvate and physiology monitoring. Hyperpolarized [2-^{13}C]pyruvate was obtained by DNP technique with OX63 trityl radical and dissolved in 4 ml of water/EDTA using a HyperSense system. Two to 2.2 ml of the hyperpolarized solution was injected into the animals. NMR experiments were performed on a horizontal 9.4T Oxford magnet equipped with a Varian INOVA console. ^{13}C spectra were acquired using pulse-acquire (flip angle 4.5° at coil center, TR = 3sec) with a surface coil positioned above the head of the animal.

Results. The enhancement factor for [2-^{13}C]pyruvate at the time of measurement was found to be 1,500 on phantom (compared to the thermal equilibrium signal). In the rat brain *in vivo*, resonances from both [2-^{13}C]pyruvate (206.71 ppm) and [2-^{13}C]pyruvate hydrate (95.57 ppm) were readily observed seconds after injection. After averaging the first 10 scans in the time course, a small signal corresponding to [2-^{13}C]lactate resonance (70.13 ppm) could also be observed (Figure 1a). The apparent T_1 was 14.6 ± 0.5 s for [2-^{13}C]pyruvate (Figure 2). This observed decay results from T_1 losses of [2-^{13}C]pyruvate, and also presumably from exchange with [2-^{13}C]lactate, which is expected to have a much shorter T_1 (~1 – 1.2 s) (4) due to the presence of the attached proton. The short T_1 of lactate might also explain the smaller signal intensity observed for this metabolite.

Discussion. [2-^{13}C]pyruvate is expected to result in the formation of [1-^{13}C]acetylCoA, which enters the TCA cycle to form [5-^{13}C]citrate and eventually [5-^{13}C]2-oxoglutarate and [5-^{13}C]glutamate. However, in the present study, we did not observe signals arising from these metabolites or any TCA cycle intermediates. Similarly, a previous study reported no signal from metabolites in the brain after infusion of hyperpolarized [1-^{13}C]acetate (5), although it is also expected to result in formation of [1-^{13}C]acetylCoA (in astrocytes). One hypothesis for this absence of signal from AcetylCoA or TCA cycle intermediates is that the hyperpolarized molecules bind to enzymes such that the T_1 would become short enough to cause complete destruction of hyperpolarization.

Conclusion. We successfully hyperpolarized [2-^{13}C]pyruvate and detected resulting hyperpolarized signals *in vivo* in the rat brain after iv injection of the compound. A small signal from [2-^{13}C]lactate was observed as a by-product of [2-^{13}C]pyruvate.

a)

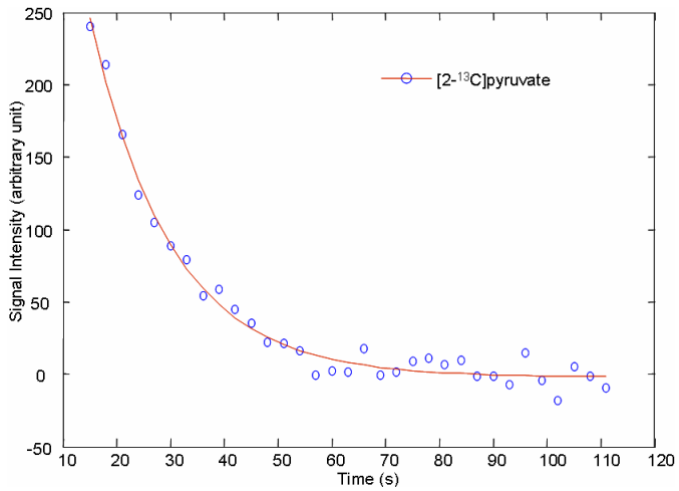


b)

Resonance	Chemical shift (ppm)
[2-^{13}C]pyruvate	206.71
[2-^{13}C]pyruvate hydrate	95.57
[2-^{13}C]lactate	70.13

Figure 1. a) Spectrum acquired in a rat (number of scans, 10, average of 30 seconds). ^{13}C-Formate signal comes from the external reference used for pulse calibration. b) Summary of chemical shifts values for observed resonances.

Figure 2. Plot of the decay of the signal of [2-^{13}C]pyruvate resonance *in vivo* (open circles) and exponential fit (red solid line).



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