In vivo real-time metabolic studies in mice at physiological concentrations following 1-^{13}C lactate injection

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Introduction

Hyperpolarized magnetic resonance spectroscopy (MRS) via dissolution dynamic nuclear polarization (DNP) allows for the observation of real-time metabolic processes *in vivo*, thus providing the opportunity to highlight the biochemical impairments linked to pathological states in various organs, including the brain [1]. One of the issues with hyperpolarized MRS is that the injection of the substrate, in particular in the case of pyruvate, often leads to supraphysiological concentrations. In the present study, we show that hyperpolarized 1-^{13}C lactate [2], which has been shown to be an essential brain energy fuel molecule [3], allows for the detection of real-time metabolism *in vivo* at physiological concentrations.

Methods

3 M sodium 1-^{13}C sodium pyruvate (Sigma-Aldrich) solution containing 33 mM TEMPO radical was prepared in a solvent mixture of D_2O / d_6-ethanol 4:1 (v/v)) and 1-^{13}C lactate solutions were prepared by mixing equal amounts (w/w) of d8-glycerol and 1-^{13}C sodium lactate solution (Sigma-Aldrich, 45%-55%)(w/w) with 33 mM TEMPOL radical. For DNP process, both samples were rapidly frozen in liquid nitrogen to form transparent droplets and then separately polarized using a custom-designed DNP polarizer operating at 5T and 1 ± 0.05K [4]. The wild type mice (n=4, with an average weight of 45g) were anesthetized using 1.5-2% isoflurane and their physiology was monitored during the experiments. Measurements were carried out on a 9.4 T/ 31 cm actively shielded animal scanner (Varian/Magnex) using home-built dual channel surface coil including 12 mm diameter quadrature ^{1}H loops and an 8 mm diameter ^{13}C surface coil. Once the samples were optimally polarized, the frozen droplets were rapidly dissolved and transferred into an infusion pump located inside the magnet bore. A volume of 500 µL (lactate) or 200 µL (pyruvate) of hyperpolarized solution was injected within 3 s in a mouse via a catheter placed in a femoral vein [4]. In all experiments data acquisition started 3 s after the dissolution, i.e. at the beginning of the infusion. Series of spectra were detected following the application of a 30° BIR4 adiabatic pulse every 3 s. Localization was achieved by placing the surface coil on top of the mouse head.

Results and Discussion

Typical time courses of substrate and metabolites signals following the infusion of 1-^{13}C pyruvate are shown in Fig.1 along with the sum of the 2nd to the 25th spectrum. In addition to the ^{13}C pyruvate signal, lactate, alanine, pyruvate hydrate and bicarbonate ^{13}C resonances were observed. The typical time courses following the infusion of 1-^{13}C lactate are displayed in Fig.2 together with the sum of the 2nd to the 25th spectrum. Lactate, alanine, pyruvate and bicarbonate signals were detected. The reactions catalyzed by lactate dehydrogenase and alanine transaminase are reversible and the ^{13}C label exchange rapidly leads to an equilibrium representative of the metabolites pool sizes [5]. The small signal intensity of the pyruvate peak following lactate injections reflects the small endogenous pool size of this metabolite. We observed that the lactate to alanine ratio was nearly identical following both the pyruvate and the lactate injections (see Table 1). At first approximation the lactate pool is thus neglibly altered by the injection and consequently all metabolites stay at physiological concentrations throughout the experiment.

Fig.1

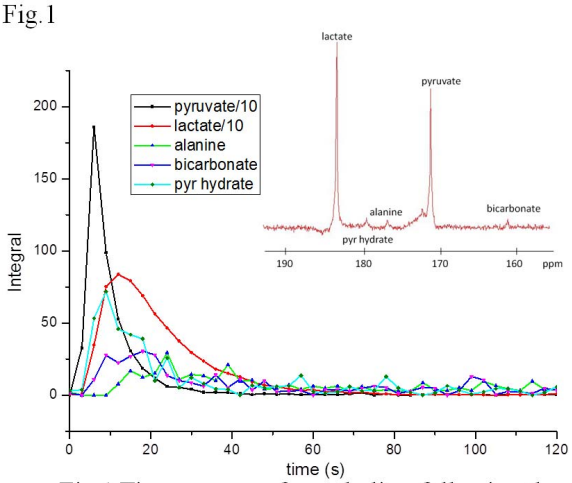

Fig.1 Time courses of metabolites following the 1-13C pyruvate injection and summed spectrum from the 2nd peak to 25th peak.

Fig.2

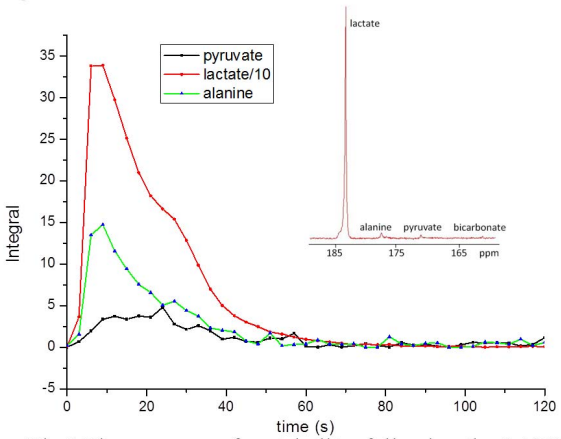

Fig.2 Time courses of metabolites following the 1-13C lactate injection and summed spectrum from the 2nd peak to the 25th peak.

Conclusions

We demonstrated that the time evolution of 1-^{13}C lactate and its metabolites pyruvate and alanine (and possibly bicarbonate) can be monitored in real time in the mouse head even when the hyperpolarized precursor is injected at physiological doses. The ability to perform *in vivo* hyperpolarized MR experiments without altering the physiology will simplify the interpretation of the metabolic studies and could open the way to potential clinical applications of hyperpolarized 1-^{13}C lactate.

hyperpolarized solution		estimated blood concentration	lactate/pyruvate (from 2nd to 25th)	pyruvate/lactate (from 2nd to 25th)	lactate(max)/ alanine (max)
1-13C pyruvate	mouse 1	4.9 mM	1.379	-	28.4
	mouse 2	5.8 mM	0.834	-	22.5
1-13C lactate	mouse 3	5.5 mM	-	0.016	19.9
	mouse 4	5.6 mM	-	0.018	23.0

Table.1 Summary of hyperpolarized 13C pyruvate and 13C lactate data

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