ESTABLISHMENT OF HYPERPOLARIZED 13C MR IN THE ISOLATED PERFUSED HEART

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Introduction

Hyperpolarization of ^{13}C-labelled metabolic substrates has been demonstrated to provide the MR signal necessary to visualize substrate uptake and metabolism in real time, *in vivo* [1]. This dynamic information could provide the basis for kinetic modelling of cellular uptake and enzymatic activity; however, understanding of the physiological interactions between the hyperpolarized substrate and tissue is necessary. The isolated perfused heart provides the ideal model system to gain this understanding, as many parameters can be easily controlled. This study demonstrated successful uptake of hyperpolarized ^{13}C by the perfused rat heart. We report acquisition of *ex vivo* spectra describing the real time uptake and metabolism of pyruvate, and have directly compared them with analogous *in vivo* results.

Methods

Hearts from 3 male Wistar rats were perfused in the Langendorff mode, and examined using hyperpolarized MR, as described below. In a separate experiment, 3 male Wistar rats were anaesthetized with isofluorane and examined using hyperpolarized MR. The resultant e*x vivo* dynamic spectra were compared with the equivalent *in vivo* data.

Isolated heart perfusion: Rats were anaesthetized with pentobarbital, and hearts were excised and arrested in heparinised buffer at 4ºC. Hearts were perfused in Langendorff mode with Krebs-Henseleit buffer plus 11 mM glucose and 2.5 mM pyruvate, and maintained at 37ºC and a constant pressure of 80 mmHg. Cardiac function was measured via an intraventricular balloon, connected to a pressure transducer. The heart was placed at isocentre of an 11.7 T vertical bore MR scanner.

MR-Protocol: 1-^{13}C-pyruvate was used for all experiments and was hyperpolarized and dissolved as previously described [1]. The resultant hyperpolarized tracer consisted of an 80 mM solution of hyperpolarized sodium 1-^{13}C-pyruvate at pH 7.2 – 7.9, with a polarization of ~30%.

Ex vivo protocol Immediately after dissolution, the tracer was diluted to 2.5 mM in standard Krebs-Henseleit buffer. The tracer (2 ml) was injected into the heart over 20 s, via a drug line maintained at 37ºC. Cardiac spectra were acquired for 2 min following injection with 1 s temporal resolution and a flip angle of approximately 5°.

In vivo protocol Immediately after dissolution, the hyperpolarized pyruvate solution was diluted to 40 mM in standard Krebs-Henseleit buffer, and 1 ml was injected over 10 s via a tail vein catheter into the anaesthetized rat. The rat was positioned in the centre of a horizontal bore 7 T MR scanner. Kinetic modelling suggests that this injection protocol results in a plasma pyruvate concentration of approximately 2.5 mM, equivalent to the perfused heart protocol. Cardiac spectra were acquired for 1 min following injection with 1 s temporal resolution and a flip angle of approximately 5°.

Data Analysis: Conversion of pyruvate to lactate, alanine, and bicarbonate was monitored and spectra were quantified in jMRUI [2]. Variations in polarization were standardized between data sets by normalizing metabolite peak area to maximum pyruvate peak area. The three *in vivo* datasets were averaged and compared to the three averaged *ex vivo* datasets in terms of metabolite/pyruvate ratio, time to peak, time to arrival, and decay rate.

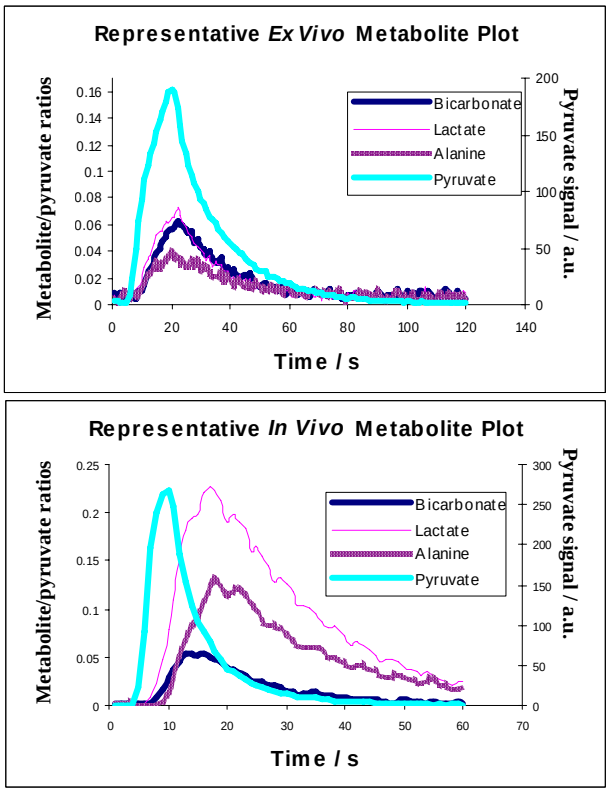

Figure 1 *Comparison between injection of 2.5 mM pyruvate into a perfused heart and in the heart* in vivo.

Results

Figure 1 shows the resultant averaged metabolite plots, from the *ex vivo* and *in vivo* experimental protocols. One obvious difference was the delay in metabolite accumulation in the living rat, as opposed to the virtually simultaneous progression of pyruvate and metabolites in the perfused heart. In both cases, metabolites appeared approximately 2-3 s after the appearance of pyruvate. However, in the perfused heart, maximum metabolite signals coincided with the pyruvate maximum, while *in vivo*, metabolites reached their peak 5-8 s after pyruvate.

The pattern of metabolite production varied considerably between the perfused heart and *in vivo* data. The bicarbonate production relative to the amount of pyruvate remained constant between the two systems. However, relative alanine and lactate production were substantially lower in the perfused heart than *in vivo* (Figure 2). This phenomenon was reflected in the relative magnitude of each metabolite; in the perfused heart bicarbonate signal was detected at the same level as lactate signal, with low levels of alanine. In the living rat, lactate and alanine were the dominant products, while bicarbonate was detected to a lesser extent.

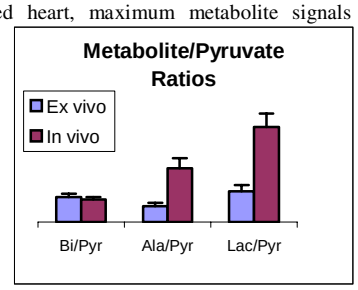

Figure 2 *Metabolite/pyruvate ratios in the* in vivo *and* ex vivo *heart.*

Discussion

The Langendorff perfusion setup itself was likely responsible for the kinetic differences between the perfused heart and *in vivo* spectra. In the perfused heart, perfusate and hyperpolarized tracer were delivered via the aorta directly into the coronary arteries, rather than entering the heart via the right atrium and traversing the cardiopulmonary system, as it would *in vivo*. Therefore, in the perfused heart, virtually all injected pyruvate was delivered immediately for myocardial utilization, and metabolite accumulation took place rapidly. The variation in relative metabolite accumulation between perfused heart and *in vivo* spectra may have resulted from increased mitochondrial pyruvate uptake and flux through pyruvate dehydrogenase (PDH), at the expense of cytosolic production of alanine and lactate (Figure 2). The absence of fatty acids in the perfusion buffer would explain the increased PDH flux and bicarbonate detection.

We anticipate that the easily manipulated Langendorff isolated perfused heart system will serve as an important tool in the quantitative modeling of normal and disordered *in vivo* myocardial metabolism with hyperpolarized substrates.

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2. Naressi, A., et al. , Computers in Biology and Medicine, 2001. **31**: p. 269-86.

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