

THE EFFECT OF HYPERPOLARIZED TRACER CONCENTRATION ON MYOCARDIAL UPTAKE AND METABOLISM

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Introduction

Hyperpolarization of ¹³C-labelled metabolic substrates provides the MR signal necessary to visualize *in vivo* substrate uptake and metabolism in real time [1]. This dynamic information could provide the basis for the kinetic modeling of cellular uptake and enzymatic activity. However, to achieve this end, an understanding of the physiological interactions between the hyperpolarized substrate and tissue is necessary. In this study, the relationship between initial pyruvate tracer concentration and the MR signal of pyruvate and its metabolic products was examined in the heart of living rats. This information could be invaluable to ascertain the tissue-specific rate and required concentrations inherent to cellular uptake of a particular hyperpolarized substrate.

Methods

Six male Wistar rats were anaesthetized and placed in a 7T MR scanner for examination using hyperpolarized MR, as described below. Hyperpolarized pyruvate solution, obtained as previously described [1], was rapidly diluted to 10 mM, 20 mM, 30 mM, or 40 mM with Krebs-Heinslet buffer, or maintained at 80 mM. Each rat was examined up to 3 times, with at least a 1 hr interval between injections, using a different concentration of hyperpolarized tracer for each examination. In total, 3 experiments were conducted at each pyruvate concentration and the data from each concentration was averaged into a single metabolite time course.

MR-Protocol: 1-¹³C-pyruvate was used for all experiments. The tracer (1 ml) was injected over 10 s via a tail vein catheter into the anaesthetized rat which was positioned in the centre of a horizontal bore 7T MR scanner. Immediately prior to injection, a ¹³C-MR pulse-acquire spectroscopy sequence was initiated. Cardiac spectra were acquired for 1 min following injection with 1 s temporal resolution and a flip angle of approximately 5°. Spectra were localized to the heart by the use of a small butterfly surface coil placed over the chest.

Data Analysis: Conversion of pyruvate to lactate, alanine, and bicarbonate was monitored and spectra were quantified in jMRUI [2]. Absolute MR signal from pyruvate and each metabolic derivative was plotted as a function of time, at each concentration. Maximum MR signal from each metabolite was plotted as a function of concentration, and fit as a first-order exponential.

Results

Following dissolution, all polarization levels were within 30 – 35%, enabling direct comparisons of absolute metabolite signal. Pyruvate signal directly depended on initial concentration. All acquired spectra were of high SNR and metabolic products were clearly visualised. At a concentration of 30 mM and below, MR signal observed from lactate, alanine and bicarbonate increased with increasing pyruvate. However, at concentrations of 40 mM and above no further increases in MR signal were observed from any metabolite. This pattern can be observed in Figure 1, which shows the bicarbonate signal over time, at each examined concentration. Maximum metabolite peak area plotted against injected pyruvate concentration showed good agreement with a first-order exponential model (Figure 2).

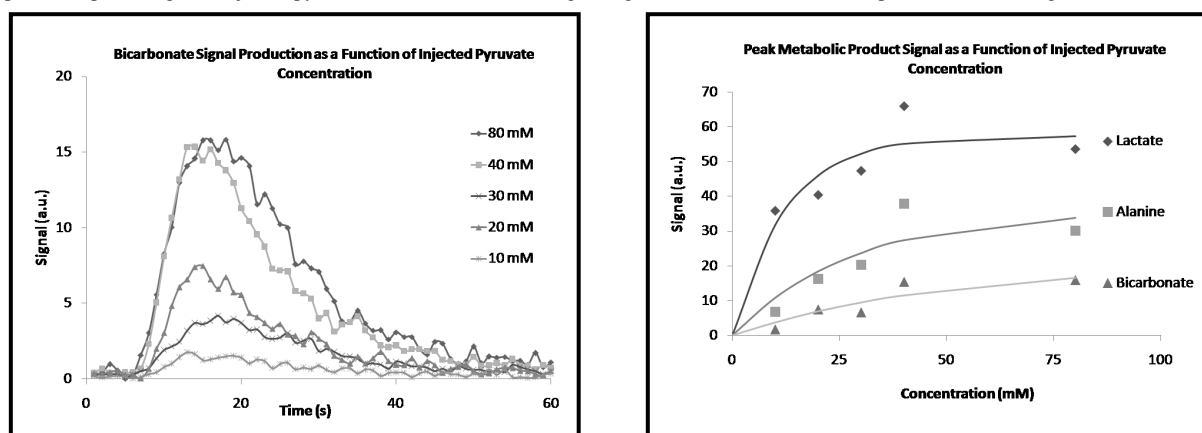


Figure 1, left Absolute bicarbonate signal over time, as a function of injected pyruvate concentration. Lactate and alanine curves varied similarly.

Figure 2, right Maximum metabolite signal, modeled as a pyruvate-concentration-dependent first order exponential function.

Discussion

Each metabolite responded similarly to changes in injected pyruvate concentration; metabolite peak area increased with increasing pyruvate up to 40 mM, with no further signal increase at the higher concentration of 80mM. This suggested that cellular uptake of pyruvate, via the monocarboxylate transporters (MCTs), was saturated at injected pyruvate concentration above 40 mM. Therefore, cytosolic pyruvate concentration reached an upper limit, and lactate, alanine and bicarbonate production was at a virtual maximum. At injected pyruvate concentrations of 30 mM and below, the amount of pyruvate transported into myocardial cells was reduced, in a manner dependent on extracellular pyruvate concentration. Thus, the reduced level of cytosolic pyruvate limited the amount of lactate, alanine and bicarbonate produced and detected.

Kinetic modelling suggests that an injected pyruvate concentration of 80 mM corresponds with an *in vivo* plasma concentration of approximately 5 mM. This work encourages a reduction of injected pyruvate concentration to 40 mM for routine studies to achieve a plasma concentration of 2.5 mM. Thus providing a pyruvate concentration closer to the physiological pyruvate concentration of 0.2 mM while still monitoring enzymatic activity that is not limited by pyruvate uptake. Further, extension of this work to novel metabolic tracer molecules could provide an important evaluation criterion for tracer utility. The most effective tracers, such as pyruvate, will not require high injected concentration to ensure rapid cellular uptake. Further analysis of this data will provide a method for non-invasive measurement of pyruvate uptake, via the MCTs. The first-order exponential model fit to each metabolite simulates Michaelis-Menten type kinetics, in which increasing pyruvate concentration exponentially increases MCT uptake, up to an asymptotic maximum. The asymptote is analogous to the V_{max} parameter measured in Michaelis-Menten kinetics.

1. Golman, K., in't Zandt, R., Thaning, M., *Real-Time Metabolic Imaging*. Proc Natl Acad Sci U S A, 2006. **103**(30): p. 11270-11275.
2. Naressi, A., et al., *Computers in Biology and Medicine*, 2001. **31**: p. 269-86.

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