Reduction of the Myocardial Intracellular Matrix as Measured by Hyperpolarized ^{13}C NMR

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Introduction

Metabolic magnetic resonance imaging using hyperpolarized (HP) ^{13}C has already been demonstrated as a promising modality for studying heart metabolism *in vivo*. The metabolic milieu characteristic of the heart is extremely complicated and must be described by at least three compartments: extra-cellular, intra-cytosolic/extra-mitochondrial, and intra-mitochondrial. To date, heart metabolism experiments using HP carbon have been performed only with [1-^{13}C]-pyruvate. Recently, this lab has studied the isolated perfused rat heart using HP-[1-^{13}C]-pyruvate after perfusion to steady state on either pyruvate or a mixture of pyruvate/octanoate. Octanoate was shown to block flux through pyruvate dehydrogenase (PDH) and prevent the appearance of HP-$^{13}CO_2$/H$^{13}CO_3^-$ typically observed in heart perfused solely with pyruvate. It was also observed that the rate of appearance of HP-[1-^{13}C]-lactate was increased in the hearts perfused with the pyruvate octanoate mixture. Using a model of the kinetics within the heart, the rate of appearance of lactate has been estimated. From these observations, we infer changes in the redox state of the heart upon perfusion with octanoate/pyruvate.

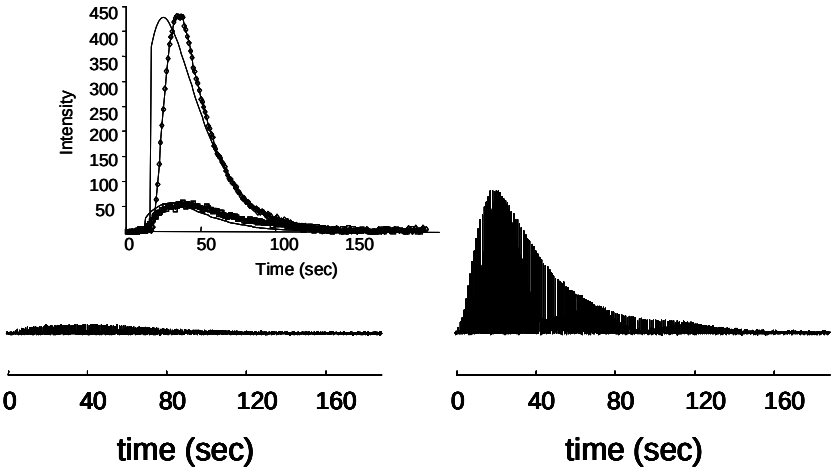

Figure 1. Time rate of appearance of lactate for perfusion with pyruvate (right) and pyruvate/octanoate (left). The inset shows a simulation of the reaction kinetics, indicating that flux into lactate is increased ~18 fold when octanoate is present.

Methods

All experiments were performed with approval by IACUC. Rat hearts were excised and immediately re-perfused in a Langendorff mode using Krebs-Henseleit (KH) bicarbonate buffer bubbled with a 95/5 mixture of O_2/CO_2 and a substrate consisting of either 2 mM natural abundance pyruvate or a mixture of 2 mM pyruvate/2 mM octanoate, both at natural abundance in ^{13}C. [1-^{13}C]-pyruvate was hyperpolarized at 3.35 Tesla (T) using an Oxford Hypersense dynamic nuclear polarization (DNP) system for ~90 minutes followed by dissolution. Three ml of solution was diluted into concentrated KH buffer and injected into the perfused heart by catheter over an average time of 90 seconds. NMR spectra were collected using an 18 mm Doty NMR probe in a Varian INOVA 14.1 T NMR system using 66 degree pulses with a 1 second acquisition time and no interpulse delay for a T_r of 1 second. Intensities of the respective NMR signals for each metabolite were obtained by integration.

Results

Figure 1 shows the plots of the lactate evolution as a function of time for both the pyruvate perfused heart and the pyruvate/octanoate perfused heart. The rate of appearance for lactate is increased ~18 fold in the case of pyruvate/octanoate perfusion. The other notable observations (not shown) are the absence of HP-CO_2/HCO_3^- production in the octanoate perfused heart and slightly increased intensity in the [1-^{13}C]-alanine signal.

Conclusions

The explanation for the increased production of lactate in the presence of octanoate in the perfused rat heart is not clear, but is likely the result of both impaired PDH activity and reduced cellular REDOX state. Flux through PDH is inhibited by simple competition with octanoate, a substrate that is preferentially oxidized by the heart. Thus intracellular pyruvate concentration is preserved and available for exchange with lactate and alanine. Additionally, the lack of regulatory mechanisms for oxidation of medium chain fatty acids leads to the abundant production of cellular NADH and decreased REDOX state. Since the equilibrium between lactate and pyruvate is REDOX dependent, increased NADH production causes the equilibrium to shift towards lactate.