Comparison of HMQC and Dynamic Nuclear Polarization for Detection of ^{13}C Tracers in the Perfused Mouse Heart

C. Harrison[1,2], A. D. Sherry[3,4], C. R. Malloy[5,6], and M. E. Merritt[7,8]

[1]Physics, University of Texas at Dallas, Richardson, TX, United States, [2]AIRC, UTSW, Dallas, TX, United States, [3]AIRC, UTSW Medical Center, Dallas, TX, United States, [4]Chemistry, University of Texas at Dallas, Richardson, TX, United States, [5]AIRC, AIRC, Dallas, TX, United States, [6]Cardiology, North Texas VA Hospital, Dallas, TX, United States, [7]Advance Imaging Research Center, UT Southwestern Med. Center, Dallas, TX, United States, [8]Radiology, University of Texas Southwestern Medical Center, Dallas, TX, United States

Introduction

Hyperpolarized [1-^{13}C]pyruvate has been shown to be a useful metabolic imaging agent for examining cardiac metabolism *in vivo* (1). The perfused mouse heart is an important metabolic model because of the large number of available knockout mice that mimic a variety of human pathologies. Intermediary metabolism can be followed in the mouse heart with short time resolution via either indirect detect proton NMR experiments with carbon-13 labeled tracer molecules (2) or using hyperpolarized ^{13}C labeled substrates. The two methods are compared here in this first demonstration of the use of hyperpolarized pyruvate for examining metabolism in the perfused mouse heart.

Methods

Mouse (C57/bl6) hearts were excised under a protocol approved by the institutional animal care and use committee. The excised hearts were immediately cannulated and perfused in a Langendorff mode with Krebs-Henseleit (KH) bicarbonate buffer bubbled with a 95/5 mixture of O_2/CO_2 and 5 mM glucose. The perfused heart was placed inside a Varian VNMRS 14.1 T spectrometer equipped with a Nalorac 8mm inverse detection probe. Two different experiments were performed on the same heart. First, 9 mL bolus of 4 mM hyperpolarized [1-^{13}C]pyruvate was injected over 20 seconds. The sample was prepared by DNP using an Oxford Hypersense system. Carbon-13 spectra were collected every 1 s using 20 degree excitation pulses.

After switching the perfusate back to 5 mM glucose, the same heart was perfused for 10 min to allow the heart to return to the steady-state before a second bolus of 9 mL of 20 mM [3-^{13}C]pyruvate was injected. The first slice of an HMQC experiment was used to detect the appearance of [3-^{13}C] labeled pyruvate, lactate, and alanine by proton detection. Two scans were used for each data point, and data points were collected every 5 seconds.

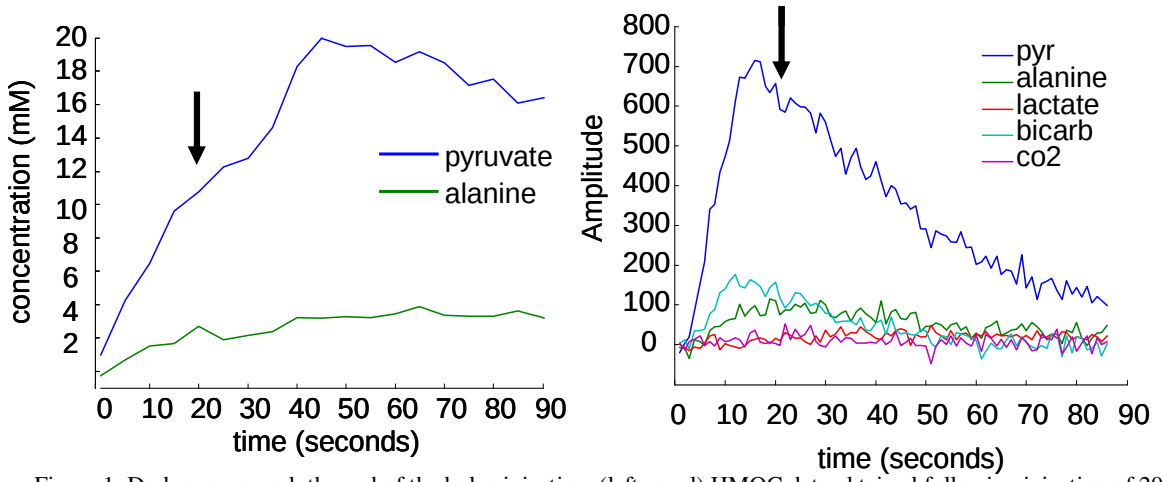

Figure 1. Dark arrows mark the end of the bolus injection. (left panel) HMQC data obtained following injection of 20 mM [3-^{13}C]pyruvate. (right panel) Carbon-13 detected signal intensity following injection of 4 mM [1-^{13}C]pyruvate.

Results

The HMQC experiment detected both [3-^{13}C]pyruvate and [3-^{13}C]alanine in the heart but lactate was not detected. The right panel shows the appearance of hyperpolarized [1-^{13}C]pyruvate and all metabolites derived from it in the subsequent carbon detect experiment. [1-^{13}C]alanine and [^{13}C]bicarbonate were the dominant metabolites detected although trace [1-^{13}C]lactate and [$^{13}CO_2$] was also visible.

Conclusions

Proton detected HMQC experiments with [3-^{13}C] pyruvate allow the flux into alanine to be measured in the mouse heart without the complicating effects of T_1 and polarization quenching via RF pulsing endemic to the hyperpolarization experiment. In contrast, the HP experiment allows the measurement of oxidative metabolism in the heart, which is not possible via HMQC. In the mouse heart, lactate production is low upon infusion of either 4 or 20 mM pyruvate. The improved signal to noise with the hyperpolarized substrate allows lower infusion concentrations to be used.

REFERENCES

1. Golman K, Petersson JS, Magnusson P, Johansson E, Åkeson P, Chai C, Hansson G, Månsson S. Cardiac metabolism measured noninvasively by hyperpolarized ^{13}C MRI. Magnetic Resonance in Medicine 2008;59(5):1005-1013.
2. Burgess SC, Babcock EE, Jeffrey FMH, Sherry AD, Malloy CR. NMR indirect detection of glutamate to measure citric acid cycle flux in the isolated perfused mouse heart. FEBS Letters 2001;505(1):163-167.