Combination of a ^{13}C cryoprobe with hyperpolarization allows real time observation of pyruvate carboxylation in the perfused mouse heart

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Target Audience

Cardiology researchers studying myocardial energy metabolism or scientists using hyperpolarized carbon-13.

Purpose

Due to a proliferation of genetically engineered mouse models of myocardial dysfunction, development of optimal methods for studying the perfused mouse heart would be scientifically advantageous. Experiments using hyperpolarized (HP) substrates have already been shown to be extremely sensitive to changes in myocardial metabolism (1). Here, hyperpolarized [1-^{13}C]pyruvate was used to study the mouse heart in a case where pyruvate carboxylation should be enhanced (high acetyl-CoA abundance). Sensitivity of the experiment was further augmented by the use of a 10 mM ^{13}C-{^{1}H} cryoprobe. This technical advance allows the observation of malate and aspartate in real time, a circumstance previously impossible in the mouse heart (Figure 1).

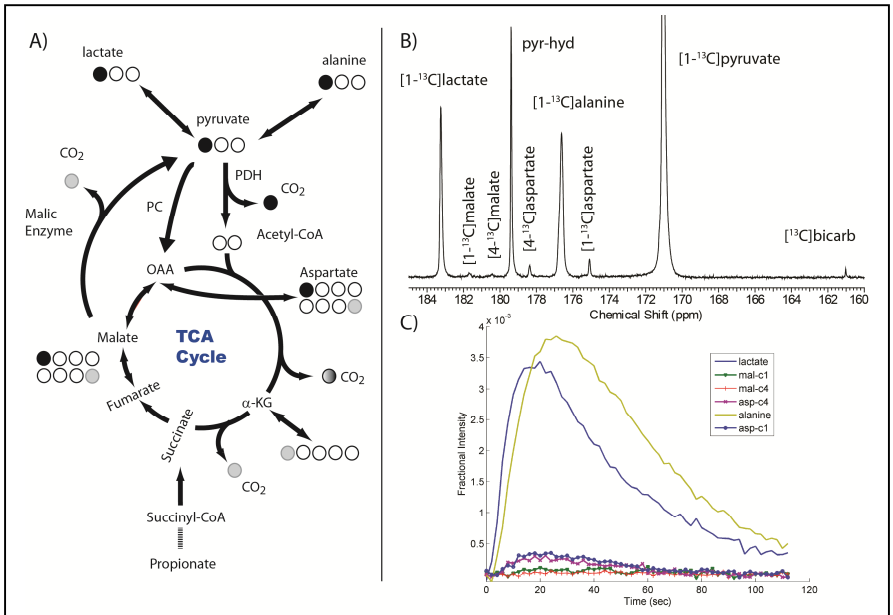

Figure 1 A) *Metabolism of [1-^{13}C]pyruvate in the myocardium. Multiple possible pathways lead to bicarbonate production.* ***B)*** *Spectra for acetate perfused mouse hearts. Note the readily observable 4-carbon metabolites that are a product of pyruvate carboxylation. If the malic enzyme or a forward turn of the TCA cycle was responsible for [^{13}C]bicarbonate production, an increase in the [4-^{13}C]malate signal would be expected.* ***C)*** *HP signals as a function of time in the perfused mouse heart. The 4-carbon metabolites each have a discernible timecourse with the enhanced sensitivity of the cryoprobe.*

Methods

A solution of trityl radical OX063 (15 mM) and ProHance® ([Gd] = 2 mM) in 10 µL of a [1-^{13}C]pyruvic acid was polarized in a HyperSense polarizer. All experiments were approved by the local IACUC. Hearts were excised from fed C57BL/6 mice under general anesthesia and perfused using standard Langendorff methods at 100 cm H_2O and 37°C. The probe was a Bruker CryoProbe optimized for ^{13}C, operating at 14.1 T, with a tuning range including ^{23}Na and ^{13}C. Hearts were shimmed on the ^{23}Na signal and the coil was then tuned to ^{13}C for the experiment.

Each heart was perfused to steady state with Krebs-Henseleit (KH) medium containing 2 mM sodium acetate and 8.25 mM unlabeled glucose during probe tuning and shimming. After ~30 min, an aliquot of HP pyruvate was quickly mixed with 20 mL of KH buffer and injected directly above the heart by catheter. Thus, the heart was exposed to the perfusate plus ~ 4 mM HP [1-^{13}C]pyruvate. ^{13}C acquisitions were initiated simultaneous with injection of HP pyruvate. All ^{13}C NMR spectra were acquired 16 ° pulses, 1 s acquisition time, and a 1 s inter-pulse delay. Relative peak areas in the phased spectra were measured by integration. The area of each metabolite resonance was normalized by the total area of the sum of all carbon signals.

Results and Discussion

In pilot experiments the SNR of the cold probe for ^{13}C was about 4x for thermally-polarized ^{13}C compared to a conventional 10 mm probe. After injection of HP [1-^{13}C]pyruvate, resonances associated with pyruvate exchange into lactate and alanine, pyruvate oxidation through PDH, and pyruvate carboxylation were observed with high sensitivity and 2 second time resolution (Figure 1 B,C). Acetate is readily oxidized by the heart, dominating the production of acetyl-CoA. In this circumstance, pyruvate carboxylation is easily monitored in real time (Figure 1C). The relative intensities of the [4-^{13}C]malate and [^{13}C]bicarbonate signals indicate that alternate pathways of CO2 production should be considered in certain perfusion conditions.

Conclusions

These results demonstrate the ease and simplicity of integrating HP ^{13}C MRS with standard cryoprobe technology. Overall sensitivity gain versus a standard ^{13}C heart perfusion with thermal polarization is estimated to be ~140,000x.

References

1. Schroeder MA, Lau AZ, Chen AP, Gu Y, Nagendran J, Barry J, et al. Hyperpolarized 13C magnetic resonance reveals early- and late-onset changes to in vivo pyruvate metabolism in the failing heart. European Journal of Heart Failure. 2013;15(2):130-40. doi: 10.1093/eurjhf/hfs192.