Detection of Sudden Changes in Cardiac Metabolism by Hyperpolarized ^{13}C MRS

Chalermchai Khemtong[1], Nicholas R Carpenter[1], Lloyd L Lumata[1], Matthew E Merritt[1], Karlos X Moreno[1], Zoltan Kovacs[1], Craig R Malloy[1], and A. Dean Sherry[1,2]
[1]*Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, TX, United States,* [2]*Department of Chemistry, University of Texas at Dallas, Richardson, TX, United States*

Introduction

Myocardial metabolism is highly flexible with alterations in substrate utilization occurring daily. The alterations were normally induced by changes in hormones, substrate availability, as well as external stimuli such as cardiac drugs. It is believed that the diseased myocardium responds differently to external stimuli from that of normal hearts. Therefore, the development of diagnostic tools that can accurately and quickly detect the changes in myocardial substrate utilization is currently of great interest. Recently, hyperpolarized (HP) magnetic resonance spectroscopy and imaging has been employed in the realm of real-time metabolism of ^{13}C-labelled substrates in heart, liver, and tumors. Due to the highly improved NMR sensitivity achieved by DNP, the metabolism of HP ^{13}C-enriched pyruvate through the citric acid (TCA) cycle by NMR and MRI has been plausible. Herein, we report the development of an HP-MRS technique to detect sudden changes in cardiac metabolism in perfused hearts as a result of cardiac drug stimulation.

Experimental Methods

A solution of trityl radical OX063 (15 mM) and ProHance® ([Gd] = 2 mM) in 4 µL [1-^{13}C]pyruvic acid was polarized in a HyperSense polarizer. Hearts were perfused using standard Langendorff methods at 100 cm H_2O pressure and 37°C. Hearts excised from male Sprague-Dawley rats were placed in an 18-mm NMR tube attached to a water-jacketed glass perfusion apparatus bubbled continuously with 95:5 O_2/CO_2 and placed inside the bore of a 14T vertical-bore magnet. Heart rate and developed pressure was monitored *via* a balloon positioned in the left ventricle. Initially, each heart was perfused with recirculating Krebs-Henseleit (KH) buffer containing 1 mM sodium pyruvate during probe tuning and shimming. After 30 min, a 3 mL aliquot of HP-[1-^{13}C]pyruvate (from a total dissolution volume of 4 mL) was quickly mixed with 20 mL of KH buffer and injected directly above the heart using a catheter. Thus, the total concentration of pyruvate experienced by each heart during the HP data acquisition was 3 mM, i.e. 2 mM HP pyruvate and 1 mM non-HP pyruvate. ^{13}C acquisitions were initiated simultaneous with injection of HP-[1-^{13}C]pyruvate and continued until all HP signals were no longer detectable. 30 s after the injection of HP pyruvate, a solution of isoproterenol (1 µM) in KH buffer was added to the perfusion chamber. All ^{13}C NMR spectra were acquired with proton decoupling using 20° pulses, 1 s acquisition time, and a 1 s delay time. The FID's were zero-filled before Fourier transformation and relative peak areas in the phased spectra were measured by integration. The area of each metabolite resonance was normalized by the total area of all metabolite resonances and plotted as a function of time.

Results and Discussion

After injection of HP-[1-^{13}C]pyruvate, resonances characteristic of HP-lactate, bicarbonate, and alanine began to appear in each spectrum as a result of pyruvate metabolism. At the time when the signal of HP-lactate had reached an apex (the maximum intensity occurred at ~18 s), isoproterenol was injected into the perfusion chamber to stimulate cardiac function. Within a few sec, the HR increased from 345 to 490 bpm and the coronary flow increased from 11 to 22 mL/min. Surprisingly, signal intensity of HP-lactate, which had begun to decay after reaching a first apex near 18 s, sharply increased in intensity and reached a second apex ~40 s later. Neither the HP-[1-^{13}C]alanine or the HP-HCO_3^- curves were altered upon addition of isoproterenol (Fig. 1B). The second apex in the HP-lactate curve was not observed in hearts undergoing the same perfusion conditions without the injection of isoproterenol (Fig. 1C). The second apex in the HP-lactate curve was subsequently traced to a sudden increase in lactate pool size arising from glycogenolysis, glycolysis and subsequent production of lactate (confirmed in separate experiments not described here). This indicates that lactate derived from glycogen rapidly mixes with the existing lactate and exchanges with any remaining HP-[1-^{13}C]pyruvate. This results in the appearance of second apex in the lactate polarization curve near 70 s (Fig. 1).

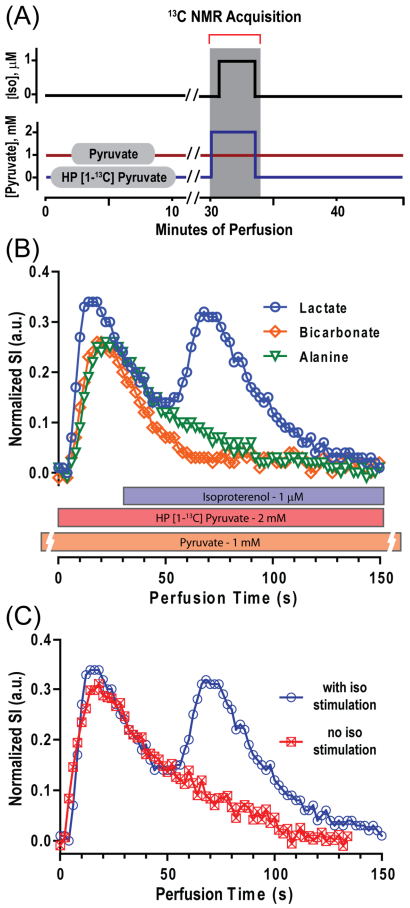

Figure 1. (A) A perfusion diagram, (B) ^{13}C NMR signals of HP-lactate, alanine and bicarbonate in a heart injected with HP-[1-^{13}C]pyruvate, (C) ^{13}C NMR signals of HP-lactate in two separate hearts with or without the injection of isoproterenol at 30 s. Perfusion with non-HP [3-^{13}C]pyruvate was for later isotopomer analyses.

Conclusions

HP ^{13}C MRS was able to detect a sudden change in cardiac metabolism of [1-^{13}C]pyruvate as a result of isoproterenol stimulation. A sharp rise in the HP-lactate curve reflects a rapid increase in lactate derived from myocardial glycogen. These results demonstrate the feasibility of using HP ^{13}C MRS as a tool to detect rapid changes in cardiac metabolism in response to exposure to cardiac drugs.

References

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