Direct Measurement of Malate-Aspartate Shuttle Activity at Different Cytosolic Redox States in Intact Hearts Using ^{13}C NMR Spectroscopy

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Introduction

The compartmentation of metabolites and enzymes between and within cytosol and mitochondria plays an important role in the regulation of energy metabolism. Although the metabolic processes in the cytosol and mitochondria are controlled locally, communication between them is important to achieve optimal substrate utilization, as well as to maintain a balance between ATP production and utilization. The malate-aspartate (M-A) shuttle, which transports cytosolic NADH into the mitochondria, provides an important mechanism to regulate metabolic activity in these two compartments. However, experimentally it has not been possible to directly measure the flux through the M-A shuttle. In this study, a novel approach that combines dynamic ^{13}C NMR spectroscopy with systems biology method of cardiac metabolism was developed to elucidate the role of M-A shuttle in the regulation of myocardial energetics. The responses of the tricarboxylic acid (TCA) cycle flux (V_{TCA}) and M-A shuttle flux ($V_{M\text{-}A}$) to augmented cytosolic redox state (NADH/NAD$^+$) induced by exogenous lactate and glucose were examined. By least-square fitting of a novel multi-domain kinetic model of cardiac metabolism to the dynamic ^{13}C NMR spectra, our findings indicate that such approach allows the direct assessment of metabolic communication between cytosol and mitochondria at various pathophysiological states.

Methods

Experimental protocols Excised 2-month-old male Sprague-Dawley rat hearts were cannulated for retrograde perfusion with a modified Krebs-Henseleit buffer. A water-filled balloon was inserted into the left ventricle through the mitral valve and connected to a pressure transducer to monitor the function of the heart. Left ventricular developed pressure (LVDP) and heart rate (HR) were measured. Myocardial O_2 consumption (MVO_2) was calculated from the coronary flow and the difference in O_2 tension between the arterial supply line and the coronary effluent. There were two experimental groups comprised of hearts perfused with either 2.5 mM [2-^{13}C] acetate alone (group 1) or 2.5 mM [2-^{13}C] acetate plus 5.0 mM glucose and 1.0 mM lactate to induce high cytosolic redox state (group 2) (n=10 for each group). After each perfusion, hearts were freeze-clamped and extracted by 7% perchloric acid for high-resolution ^{13}C NMR spectra.

Dynamic ^{13}C NMR measurements of perfused hearts ^{13}C NMR spectra were collected on a Bruker 400 MHz vertical bore spectrometer with hearts situated in a 20 mm broadband NMR probe equipped with a proton decoupling coil. Sequential ^{13}C spectra were acquired at 101 MHz with a 45° pulse angle, 0.58 s acquisition and 1 s relaxation delay over 32 scans (1 min period) until steady-state enrichment was reached. The free induction decay (FID) was acquired with an 8192 data set. A spectrum of ^{13}C natural abundance was acquired from each heart for background subtraction before the perfusion buffer was switched to ^{13}C-label substrate. Signal intensity was determined by fitting the resonance peaks with Lorentzian curves followed by the integration of the peak areas using a commercialized software MestRec (Mestrelab Research SL, Santiago de Compostela, Spain). Peak assignments were referenced to the well documented glutamate signals.

High-resolution ^{13}C NMR spectra of heart extracts *In vitro* high-resolution ^{13}C NMR spectra of heart extracts reconstituted in 0.5 mL of D_2O were acquired with a 5 mm ^{13}C/^{1}H probe inside a Bruker 900MHz Spectrometer from 1024 scans (90° pulse, 0.26 s acquisition and 4 s delay) with broadband decoupling. The FID was acquired with an 8192 data set and zero filled to 16000 to improve digital resolution. The fractional enrichment of acetyl-CoA (F_c) and the ratio of anaplerotic to TCA cycle flux (y) were determined from the multiplet structure of glutamate resonance peaks (1).

Kinetic model and analysis A comprehensive multi-domain kinetic model of cardiac metabolism was expanded from a previous model (2) by including all the mechanisms associated with the M-A shuttle, viz., redox and transamination reactions in both cytosol and mitochondria, as well as carrier-mediated transporters. The pre-steady-state ^{13}C enrichment of the 2- and 4-carbon of each metabolite was described by a set of 16 differential equations with 18 compartments in total. ^{13}C label entering the TCA cycle from acetyl-CoA pool was treated as a step function with the assumption that acetyl-CoA enrichment from ^{13}C-labeled acetate was rapid. V_{TCA} and $V_{M\text{-}A}$ were determined from the least-square fitting of the model to the ^{13}C enrichment curves of the glutamate labeling at 4- and 2-carbons.

Results

The rate pressure product (RPP) was similar for hearts at low and high cytosolic redox states (24,200±4,100 and 24,800±2,400 mmHg/min, respectively). MVO_2 (μmol/min/g wet weight) was also similar at 3.3±0.3 with acetate alone and 3.2±0.5 with acetate plus glucose and lactate. The F_c and y values determined from the analysis of *in vitro* ^{13}C spectra of tissue extracts were F_c=0.92±0.03, y=0.05 for group 1, and F_c=0.76±0.03, y=0.25 for group 2. Figure 1a shows representative, dynamic ^{13}C NMR spectra from a rat heart perfused with ^{13}C-labeled acetate. ^{13}C glutamate enrichment curves and results from least-square fitting were shown in Figure 1b. There was no significant difference in V_{TCA} between the two groups (1.07 and 0.99 μmol/min/g wet weight, respectively). However, a 38% increase in $V_{M\text{-}A}$, from 0.53 to 0.73 μmol/min/g wet weight, was observed in group 2, suggesting enhanced M-A shuttle flux associated with high cytosolic redox state induced by glucose and lactate.

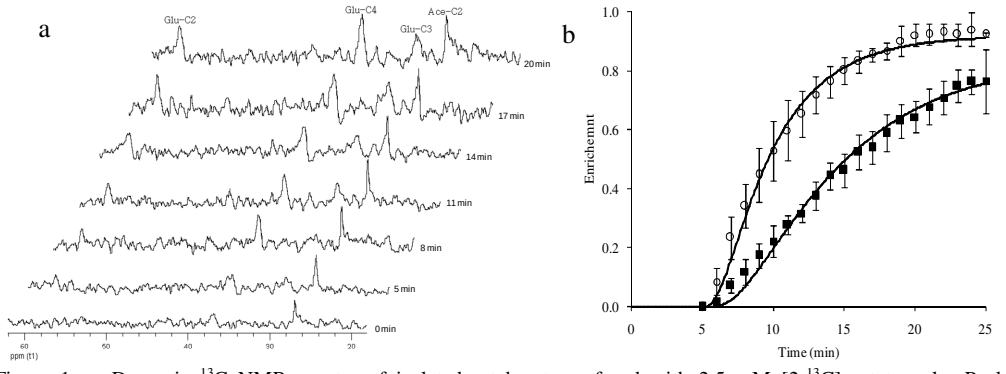

Figure 1. a. Dynamic ^{13}C NMR spectra of isolated rat hearts perfused with 2.5 mM [2-^{13}C]acetate only. Peak assignments: Glu-C2, 2-carbon of glutamate; Glu-C4, 4-carbon of glutamate; Glu-C3, 3-carbon of glutamate; Ace-C2, 2-carbon of acetate; b. Time course of glutamate ^{13}C enrichment at low cytosolic redox state. Open circles: glutamate 4-carbon; closed squares: glutamate 2-carbon. Solid lines were the model simulations with the optimal V_{TCA} and $V_{M\text{-}A}$.

Conclusion

Kinetic analysis of ^{13}C incorporation into glutamate pool showed that V_{TCA} is unaltered with changes in cytosolic redox state, whereas $V_{M\text{-}A}$ is more sensitive to alterations in cytosolic NADH/NAD$^+$. Therefore, the application of a novel multi-domain kinetic model of cardiac metabolism to the analysis of dynamic ^{13}C NMR spectroscopy data provides the opportunity to directly quantify metabolic communication between subcellular compartments via the M-A shuttle.

References

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