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Regulation of Cytosolic and Mitochondrial Oxidation via Malate-Aspartate Shuttle: An Observation Using Dynamic ^{13}C NMR Spectroscopy

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Abstract

The malate-aspartate (M-A) shuttle provides an important mechanism of metabolic communication between the cytosol and the mitochondria. In this study, dynamic ^{13}C NMR spectroscopy was combined with a multi-domain model of cardiac metabolism for direct quantification of metabolic fluxes through the tricarboxylic acid (TCA) cycle (V_{TCA}) and the M-A shuttle (V_{M-A}) in intact heart. The sensitivity of this approach to altered M-A shuttle activity was examined at different cytosolic redox states. Dynamic ^{13}C NMR spectra were acquired from isolated rat hearts perfused with ^{13}C labeled fatty acid at either low (fatty acid only) or high cytosolic redox state induced by exogenous glucose and lactate. V_{TCA} and V_{M-A} were determined by least-square fitting of the model to NMR data. Our results showed that while V_{TCA} was similar, V_{M-A} increased by 75% at high cytosolic redox state. Therefore, our proposed method provides the opportunity for direct quantification of metabolic communication between subcellular compartments via the M-A shuttle.

25.1 Introduction

The malate-aspartate (M-A) shuttle plays an important role in regulating cardiac energy metabolism by transporting cytosolic ATP-yielding reducing equivalents, i.e., NADH, indirectly into the mitochondria via a transport-transamination-redox cycle that spans the cytosolic and mitochondrial domains (1). Changes in M-A shuttle activity and shuttle-associated protein expression have been indicated at altered pathophysiological conditions. However, experimental measurements of the M-A shuttle activity have been challenging. It has not been possible to directly measure the M-A shuttle activity in intact tissues due to limitations in quantifying cytosolic and mitochondrial metabolites experimentally. To date, most investigations on M-A shuttle have employed the isolated mitochondrion system. However, such an approach has the inherent limitation of an artificial cytosolic environment.

In the last twenty years, ^{13}C NMR spectroscopy has become a valuable tool for the evaluation of cardiac metabolism (2). The utilization of ^{13}C -enriched substrates provides unique observation about tracer distribution in intermediary metabolites (3). Dynamic ^{13}C NMR observation of glutamate labeling in intact hearts has been demonstrated to be sensitive to both tricarboxylic acid (TCA) cycle flux and the M-A shuttle activity (4). Kinetic analysis methods have also been developed for quantification of metabolic fluxes from dynamic ^{13}C NMR spectroscopy data (4). Previous models have used a lumped parameter to represent the interconversion rate between glutamate and α -ketoglutarate as an index of the M-A shuttle activity (4). However, these single domain models do not provide direct quantification of the M-A shuttle flux and the distribution of shuttle-associated metabolites between the cytosol and the mitochondria.

In the present study, a novel approach that combines dynamic ^{13}C NMR spectroscopy with a multi-domain kinetic model of cardiac metabolism was developed to elucidate the role of M-A shuttle in regulating myocardial energetics. The responses of TCA cycle flux (V_{TCA}) and M-A shuttle activity ($V_{\text{M-A}}$) to augmented cytosolic redox state (NADH/NAD^+) induced by exogenous lactate and glucose were examined. Our findings indicate that such an approach allows direct assessment of metabolic communication between the cytosolic and mitochondrial compartments via the M-A shuttle.

25.2 Methods

25.2.1 Isolated heart perfusion

Hearts were excised from 2-month-old male Sprague-Dawley rats and retrogradely perfused at 100 cm hydrostatic pressure with a modified Krebs-Henseleit buffer equilibrated with 95% O_2 /5% CO_2 (pH 7.4). Perfusate was maintained at 37°C . A water-filled latex balloon was inserted into the left ventricle to monitor the contractile parameters of the heart. Left ventricular developed pressure (LVDP) and heart rate (HR) were continuously recorded. Rate pressure product ($\text{RPP}=\text{LVDP}\times\text{HR}$) was used as an index of mechanical work. Myocardial O_2 consumption rate (MVO_2) was calculated from the coronary flow and the difference in O_2 tension between the arterial supply line and the coronary effluent.

Isolated hearts were perfused with either short-chain (2.5 mM $[2\text{-}^{13}\text{C}]$ acetate, group 1, $n = 8$) or long-chain (0.32 mM $[\text{U-}^{13}\text{C}]$ palmitate with 3% albumin, group 2, $n=6$) fatty acids. A 3rd group comprised of hearts perfused with 2.5 mM $[2\text{-}^{13}\text{C}]$ acetate with additional unlabeled glucose (5.0 mM) and lactate (1.0 mM) to induce high cytosolic redox state ($n=8$). At the start of the perfusion protocol, the heart was perfused with unlabeled substrates for 15 min to establish metabolic equilibrium. A ^{31}P spectrum and a natural abundance ^{13}C spectrum were collected. Once the perfusate was switched to ^{13}C -enriched buffer, acquisition of dynamic ^{13}C NMR spectra began immediately for over 40min until steady-

state enrichment was reached. At the end of the perfusion protocol, hearts were freeze-clamped and extracted by 7% perchloric acid for quantification of substrate utilization and anaplerosis by high-resolution ^{13}C NMR spectroscopy as previously described (4). Glutamate content was measured by spectrophotometric assays.

25.2.2 NMR spectroscopy

NMR spectra of isolated perfused hearts were acquired on a Bruker 9.4T vertical-bore spectrometer with hearts situated in a 20 mm broadband NMR probe. ^{31}P spectra were acquired at 161.9 MHz with a 90° pulse angle, 1.3 s acquisition, 1 s relaxation delay, and 32 averages. Proton-decoupled ^{13}C NMR spectra were acquired at 101 MHz with 45° rf pulses, 0.58 s acquisition, 1 s relaxation delay, and 8k data points. For the acetate (group 1 and 3) and palmitate (group 2) groups, ^{13}C spectra were collected over 32 and 100 scans, respectively. Natural abundance ^{13}C signal was digitally subtracted, and the raw NMR signal was processed by exponential filtering with a line broadening of 20 Hz to enhance the signal-to-noise ratio, followed by Fourier transformation. Peak assignments were referenced to the known resonance of 2-carbon acetate at 24.1 ppm (groups 1 and 3) or triglyceride at 30 ppm (group 2). Signal intensity, representative of metabolite content, was determined by the integration of the peak areas. The time courses of fractional enrichment of glutamate at 4- (C4) and 2-carbon (C2) positions were obtained by normalizing signal intensity to steady-state enrichment of glutamate C4 measured from high-resolution ^{13}C NMR analysis of tissue extracts described below.

High-resolution ^{13}C spectra of heart extracts were acquired on a Bruker 900 MHz spectrometer with a 5 mm $^{13}\text{C}/^1\text{H}$ probe. Proton-decoupled spectra were collected over 1024 scans with 90° rf pulses, 4.26 s repetition time and 8k data set. Fractional enrichment of acetyl-CoA (F_c), i.e., the steady-state enrichment level of glutamate C4, and the ratio of anaplerotic flux to V_{TCA} (y) were determined from the multiplet structure of glutamate resonance peaks (2).

25.2.3 Kinetic analysis and statistical evaluation

In the current study, a previous multi-domain model of cardiac metabolism (5) was expanded to incorporate the dynamic labeling of major metabolite pools with ^{13}C for quantification of V_{TCA} and V_{M-A} (Figure 1). With the exception of glutamate, metabolite concentrations were taken from literature on hearts perfused with similar substrates (4; 5). V_{TCA} and V_{M-A} were determined from the least-square fitting of the model to the ^{13}C enrichment curves of glutamate C4 and C2, with the standard deviation calculated from the Jacobian matrix of the model. Simulations and parameter optimization were performed by solving the model equations using DLSODE, a robust implicit integrator for stiff and sparse systems, and NL2SOL, a solver for nonlinear least-square problems.

All measurements of this study are presented as mean $\pm$ SD. Mean values from different groups were compared by one-way analysis of variance. If there were statistical differences, multiple pairwise comparisons were performed using Tukey's test. P values less than 0.05 were considered statistically significant.

25.3 Results

25.3.1 Physiological function, glutamate content

As shown in Table 1, there were no statistical differences between the three groups in heart weight, RPP and MVO_2 . Compared to the other groups, tissue glutamate content from hearts oxidizing palmitate was significantly lower.

25.3.2 Fatty acid utilization

At low cytosolic redox state, acetate (group 1) and palmitate (group 2) contributed to $92 \pm 3\%$ and $88 \pm 5\%$ of oxidative metabolism, respectively ($p=\text{n.s.}$). At high cytosolic redox state (group 3), the contribution of acetate to oxidative metabolism was significantly reduced to $78 \pm 5\%$ due to the oxidation of exogenous unlabeled glucose and lactate via pyruvate dehydrogenase.

25.3.3 Dynamic ^{13}C NMR spectra

Representative dynamic ^{13}C NMR spectra from each group are shown in Figure 2. Compared to the high cytosolic redox state (Figure 2C), hearts at a low redox state showed delayed incorporation of ^{13}C into the glutamate pool (Figure 2A&B). ^{13}C enrichment curves of glutamate and the results of kinetic analysis are shown in Figure 3. The average half time, i.e., the time to 50% steady-state enrichment, for glutamate C4 was 6.5 min for group 1, 7.6 min for group 2, and 4 min for group 3, indicating a faster labeling kinetics for group 3.

25.3.4 Determinations of TCA cycle flux and M-A shuttle activity

Least-square fitting of the kinetic model to the glutamate labeling data is shown in Figure 3 for all three groups. At low cytosolic redox state, both V_{TCA} and $V_{\text{M-A}}$ were similar between group 1 and 2 (Figure 4). At high cytosolic redox state (group 3), V_{TCA} was unaltered compared to the other two groups. However, $V_{\text{M-A}}$ ($0.7 \pm 0.1 \mu\text{mol/min/g wt}$, $p < 0.05$) increased by 40% and 75% compared to group 1 and 2, respectively. As a result, this suggests enhanced M-A shuttle flux associated with elevated cytosolic redox state (Figure 4).

To test whether the changes in glutamate labeling kinetics in group 3 were due to lower fractional enrichment of acetyl-CoA, model simulation of glutamate enrichment in group 3 was performed by increasing the acetyl-CoA enrichment to that of group 1 using the same flux parameters obtained from group 3. With a hypothetical acetyl-CoA enrichment of 92%, simulated glutamate enrichment curves reached a steady-state level similar to that of group 1. However, glutamate labeling kinetics was faster than that of group 1. Therefore, the kinetics of ^{13}C enrichment in glutamate is determined by V_{TCA} and $V_{\text{M-A}}$ instead of the fractional acetyl-CoA enrichment level.

25.4 Discussion

Previous kinetic analysis methods used a lumped parameter as an index of the inter-conversion rate between glutamate and α -ketoglutarate (4). Distribution of metabolites between mitochondria and cytosol was not considered in these earlier studies. Therefore, quantification of M-A shuttle activity was not complete. In this study, we developed a multi-domain ^{13}C labeling model of cardiac metabolism with detailed representation of the M-A shuttle. By least-square fitting of the model to the dynamic NMR-measured glutamate enrichment, we found an increase in M-A shuttle activity induced by elevated cytosolic redox state in intact hearts. Thus, we show here the first approach that allows the direct assessment of metabolic communication between cytosol and mitochondria via the M-A shuttle.

Compared with hearts perfused with fatty acid only (group 1 and 2), high cytosolic redox state (group 3) induced a shift of oxidative metabolism, i.e., oxidation of glucose and lactate reduced the contribution of acetate to the total oxidative metabolism. Like in the brain (6), ATP production in the heart is also tightly coupled to energy demand, i.e., the workload. Since high cytosolic redox state did not induce an increase in workload, both oxygen consumption and the TCA cycle flux were unaltered. However, exogenous lactate and

glucose led to an accelerated incorporation of ^{13}C into the glutamate pool, suggesting an enhanced M-A shuttle activity.

In conclusion, kinetic analysis of ^{13}C incorporation into the glutamate pool showed that TCA cycle flux is unaltered with changes in cytosolic redox state, whereas the M-A shuttle activity is more sensitive to alterations in cytosolic NADH/NAD⁺. Our proposed method provides the opportunity to directly quantify metabolic communication between subcellular compartments via the M-A shuttle in intact, functioning hearts.

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Fig. 25.1.

Compartmental model of cardiac metabolism. *, ^{13}C labeled species. Large boxes represent metabolite pools. Small boxes represent isotopic compartments for modeling.

Fig. 25.2. Dynamic ^{13}C NMR spectra of isolated rat hearts perfused with 2.5 mM $[2\text{-}^{13}\text{C}]$ acetate (A), 0.32 mM $[\text{U-}^{13}\text{C}]$ palmitate (B), and 2.5 mM $[2\text{-}^{13}\text{C}]$ acetate plus unlabeled 5.0 mM glucose and 1.0 mM lactate (C). Peak assignments: GLT-C2, glutamate C2; GLT-C4, glutamate C4; GLT-C3, glutamate C3; ACE-C2, acetate C2; TRI, triglycerides.

Fig. 25.3. Time course and model fitting of glutamate ^{13}C enrichment. A. group 1; B. group 2; C. group 3. Open circles: GLT-C4; closed squares: GLT-C2. Solid lines were the model simulations with optimal V_{TCA} and V_{M-A} .

Fig. 25.4.

V_{TCA} and V_{M-A} . LRX ACE: low cytosolic redox state, acetate perfusion; LRX PAL: low cytosolic redox state, palmitate perfusion; HRX: high cytosolic redox state. *: $p < 0.05$.

Table 25.1

Physiological function and tissue glutamate content

	Low cytosolic redox state		High cytosolic redox state
	Group 1	Group 2	Group 3
Perfusate	¹³ C-acetate	¹³ C-palmitate	¹³ C-acetate+glucose+lactate
Heart Weight (g)	1.42±0.23	1.33±0.18	1.48±0.17
RPP×10 ³ (mmHg/min)	20.9±5.3	16.6±3.8	18.8±5.0
MVO ₂ (μmol/min/g wt)	2.5±0.5	2.4±0.5	2.6±0.6
Glutamate (μmol/g wt)	3.4±0.5	2.5±0.4*	3.2±0.7

* significant difference, $p < 0.05$.