Malic Enzyme: A Significant Player in Cardiac Metabolism

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Introduction

The tricarboxylic acid (TCA) cycle plays a central role in energy production in the heart. In addition, it also provides biosynthetic intermediates for many anabolic pathways. Thus, in order to prevent impairment of the TCA cycle, cataplerotic pathways (which deplete TCA cycle intermediates by using them as substrate for biosynthesis of other molecules) need to be balanced with anaplerotic pathways (which increase TCA cycle intermediates by providing entry of substrates at sites other than through acetyl-CoA) so that mitochondrial concentrations of the intermediates remains constant. The existence of this enzyme has been well established in many tissues [1]. There has also been indirect evidence of malic enzyme activity, with the direction of the net flux dependent upon intracellular redox state and/or substrate concentrations [2]. Significant activity of this pathway could confound analysis of ^{13}C-NMR spectra. In this report, we provide direct evidence for significant activity of this pathway in isolated perfused rabbit hearts.

Purpose

To determine if ^{13}C-NMR spectroscopy can be used to quantitate malic enzyme activity under controlled conditions in the isolated perfused rabbit heart.

Methods

Hearts (n=20) were isolated from New Zealand White male rabbits (1.5-2 kg) and perfused at 37°C in a modified Langendorff preparation. Buffer was a modified Krebs Henseleit solution containing 3% bovine serum albumin, 0.7 mM palmitate, 0.2 mM β-hydroxybutyrate, and 5 mM glucose as metabolic substrates. Insulin was added to the buffer (10μU/mL) to aid glucose uptake. Perfusion pressure was maintained at 75 mmHg, and a balloon was placed into the left ventricle for continuous monitoring of developed pressures. Following an equilibration period (~30 minutes) for physiological parameters to stabilize, hearts were switched to a buffer containing ^{13}C-labeled substrates (2,4-^{13}C-β-hydroxybutyrate, U-^{13}C-palmitate), and perfused for 2,4,6,8,10,15,30,or 45 minutes. Hearts were rapidly frozen with liquid nitrogen cooled tongs, and extracted using perchloric acid. ^{13}C-NMR spectroscopy was performed on the extracts and isotopomer analysis was used to calculate the fractional enrichments of glutamate.

Results

The concentration of the TCA cycle intermediates is below the levels required for isotopomer analysis using ^{13}C NMR spectroscopy. Thus, enrichment patterns of glutamate, aspartate and alanine (which are in rapid exchange with α-ketogluterate, malate and pyruvate respectively, and are present in detectable concentrations) are used to reflect the TCA turnover in isotopomer analysis using ^{13}C spectroscopy.

The substrates presented to the heart in the perfusion buffer (2,4-^{13}C-β-hydroxybutyrate, U-^{13}C-palmitate) will result in the generation of acetyl Co-A that is labeled in the C_2 position, or both the C_1 and C_2 positions. ^{13}C-2-acetyl Co-A should not enrich the C_5 of glutamate, and labeling pattern due to 1,2-^{13}C-acetyl Co-A should be reflected in the C_5 position of glutamate by the appearance of a doublet. Also, given the introduction of the label only as acetyl Co-A should not result in the enrichment of alanine unless there is anabolic flux from the TCA to pyruvate. The analysis of the obtained spectra revealed the occurrence of a C_5 singlet in glutamate and corresponding ^{13}C enrichment in the C_4 position of aspartate and in the C_2 position of alanine. The C_5 singlet of glutamate arises from C_1 enriched acetyl Co-A. Upon review of existing literature and computer simulation models it was concluded that the occurrence of these specific enrichments is due to flux from malate to pyruvate through malic enzyme/PEPCK, which would yield 2-^{13}C-pyruvate which is subsequently metabolized to 1-^{13}C-acetyl Co-A. The presence of the C_5 singlet was measured against the presence of the C_5 doublet, and the fractional enrichment calculated, to ensure that we were well above the levels of natural abundance.

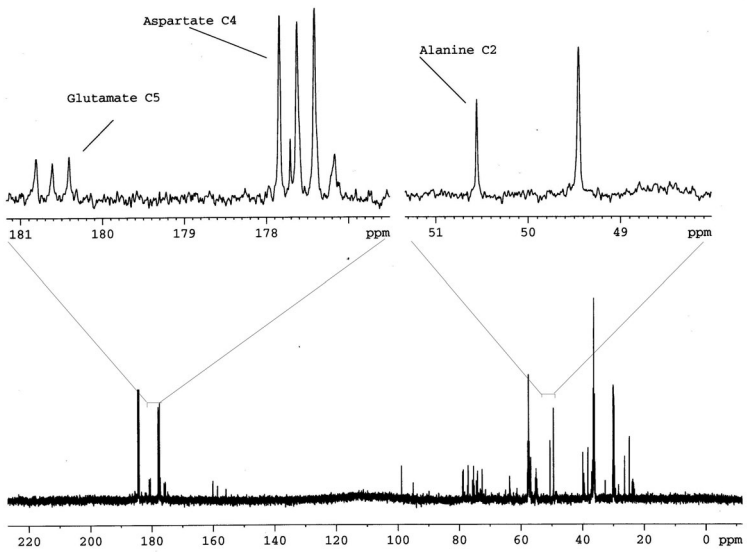

Figure 1: Representative ^{13}C spectra from isolated perfused rabbit heart showing significant enrichment at the C_5 singlet of glutamate, the C_4 position of aspartate and the C_2 position of alanine.

Conclusions

Under these perfusion conditions, flux through malic enzyme in the rabbit heart was significant; estimated at ≥25% of the total TCA cycle turnover. Traditional estimates of anaplerotic flux (using the ratio of the steady-state ^{13}C enrichments in the C3 (or C2) and C4 positions of glutamate) do not reflect this cataplerotic pathway, since anaplerotic flux from aspartate is also labeled. ^{13}C NMR spectroscopy can be used to quantitatively measure malic enzyme activity by careful selection of labeled substrates for perfusion along with concomitant observation of the glutamate C_5 singlet, and the subsequent enrichment patterns of alanine and aspartate.

Refrences:

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2. Sundqvist, K et al. Biochem. J. 243(3): 853-7, 1987.