Dynamical *In Vivo* ^{13}C NMR Spectroscopy for the Assessment of Myocardial Metabolic Fluxes

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ABSTRACT

Dynamical *in vivo* ^{13}C NMR spectroscopy was used to assess metabolic fluxes of myocardial carbon metabolism in anesthetized rats. Metabolic fluxes were calculated from enrichment of myocardial metabolites using two different methods proposed originally for isolated hearts and adapted here to the *in vivo* situation. Accordingly, myocardial tricarboxylic acid cycle flux (V_{TCA}) *in vivo* was estimated at 1.26 ±0.13 and 1.34±0.07 µmol/min/g wet weight (mean±SEM, n=5) using a simple indexing method and a kinetic model based on coupled differential equations, respectively. Advantages of both methods for *in vivo* use are discussed.

INTRODUCTION

The tricarboxylic acid (TCA) cycle is the major source of energy provision in the heart and is thus regarded as pivotal determinant for contractile function.[1] Currently, new drugs are being evaluated, which aim at optimizing substrate metabolism during myocardial injury.[2] However, metabolic fluxes are difficult to be analyzed and measured in the intact organism. For isolated hearts, two common methods have been established, i.e. TCA cycle indexing[3] and kinetic modeling[4] based on time difference measurements between the glutamate labeling in C4 and C3, and coupled differential equations, respectively. Nonetheless, controversy has arisen as to the accuracy of the methods with respect to ill-defined factors such as exchange rates and pool sizes.[6,7]

Here, we report that dynamical *in vivo* ^{13}C NMR spectroscopy can be used as a basis for assessing myocardial metabolic fluxes *in vivo*. Myocardial TCA cycle flux *in vivo* was determined with adapted variants of the two models proposed previously flux analysis for perfused hearts. The performance and the results of the two methods were evaluated with respect to their usability in the *in vivo* situation.

MATERIALS AND METHODS

^{13}C NMR spectroscopy at 7 Tesla (75 MHz) was performed on anaesthetized rats using a Bruker Biospec 70/20 spectrometer equipped with a surface coil (Ø 29 mm) for signal excitation and reception. Blocks of 300 scans (6 min) were recorded in the pulse-acquire mode using an adiabatic 90° BIR pulse (T_p= 2 ms, $\Delta\Omega_{max}\cdot T_p$= 100, tan(κ)= 10), a spectral width of 250 ppm, and 4k data points. Broadband proton decoupling during the acquisition and NOE were effectuated with a whole body ^{1}H resonator. Signals from superficial tissues were minimized by saturation slices (hermite excitation, hyperbolic increase of pulse amplitude) in the ^{13}C frequency domain which were placed on the chest wall. All acquisitions were respiration- and ECG-gated. Myocardial metabolism was assessed during concomitant infusions of non-radioactive [^{13}C] isotopomers of glucose, 3-hydroxybutyrate, and acetate. Metabolic fluxes were derived from the progressive label incorporation into myocardial metabolites using 2 different mathematical methods.[3,4]

RESULTS AND DISCUSSION

Indexing TCA cycle flux via half-maximum enrichment of glutamate. TCA cycle turnover was estimated using the indexing method of successive glutamate enrichment proposed previously.[3] The time difference (Δt_{50}) between half-maximum enrichment of glutamate C4 and C3 was 11.4 min (Fig. 1). The TCA cycle flux parameter (K_T)[3] was thus

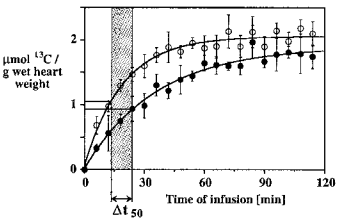

Fig. 1. Time course of ^{13}C enrichment of myocardial glutamate C4 (○) and C3 (●) as obtained by integration of Lorentzian lines fitted to the individual resonances of dynamical *in vivo* ^{13}C NMR spectra (mean±SEM, n=5).

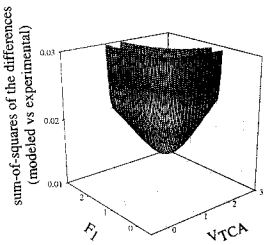

Fig. 2. Convergence of the kinetic model and experimental *in vivo* ^{13}C NMR data as a function of metabolic fluxes.

estimated at 0.88 ± 0.09 µmol/min/g wet weight for hearts *in vivo* exerting an average heart rate-pressure product (RPP) of 27,480±669 mmHg/min (mean ± SEM; n=5). Absolute TCA cycle flux rates were calculated from the flux parameters by allowing for the relative contribution of anaplerosis.[5] Accordingly, myocardial TCA cycle flux *in vivo* was estimated at 1.26 ±0.13 µmol/min/g wet weight.

Least-square fitting of a kinetic model to in vivo NMR data. In this model, labeling of myocardial metabolites is described by nine coupled differential equations defining the flux through each metabolite pool. The model originally proposed for analysis of metabolism in isolated perfused hearts[4] was expanded to account for the *in vivo* synthesis of myocardial glutamine and the infusion kinetics of the substrates. In addition to the tracer dynamics, this model requires the pool sizes of myocardial metabolites as input parameters which were measured in tissue extracts. Best correspondence of the kinetic model and the experimental data (simplex algorithm, flux variation from 0-2.5 in steps of 0.01) was found for V_{TCA}= 1.34±0.07 µmol/min/g wet weight.

The exchange rate between α-ketoglutarate and glutamate (F_1) was estimated at 1.22±0.08 µmol/min/g wet weight and thus was similar to V_{TCA} (Fig. 2). This is in agreement with findings in normal isolated hearts.[4,7] In contrast, however, a marked synthesis of glutamine of V_{gln} = 0.14±0.02 µmol/min/g wet weight was observed *in vivo* which has not been reported for isolated perfused hearts.[3,4]

Moreover, since the relative contributions of substrate for oxidative metabolism are known from the ^{13}C-NMR data, myocardial oxygen consumption could be calculated.[8] Myocardial oxygen consumption was thus estimated at 2.88 ± 0.14 µmol/min/g wet weight.

Comparison of the models. Both methods provided equal TCA cycle fluxes within the limits of statistical significance (p = 0.57, unpaired t-test). The method of determining V_{TCA} via glutamate Δt_{50} has the advantage of being straightforward, particularly for *in vivo* use, and is relatively independent of metabolite pools not detected by ^{13}C NMR spectroscopy. The advantage of the kinetic model is, that it provides additional metabolic parameters.

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