Myoglobin - a switch for cardiac substrate selection

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Introduction

Myoglobin (Mb) is generally considered to be an important intracellular O_2 binding hemoprotein found in the cytoplasm of vertebrate type I and IIa skeletal and cardiac muscle tissue. Previously, we have shown that disruption of Mb in mice (myo$^{-/-}$) induces multiple adaptative changes all aimed at steepening the O_2 gradient from the capillary to mitochondria, e.g. increased coronary flow and capillary density (1,2). This study explored whether these alterations are accompanied by changes in metabolism using PET, MRI, ^{13}C NMR spectroscopy, and proteomics of hearts from Mb knockout (myo$^{-/-}$) mice recently generated in our laboratory (1).

Methods

MR investigations were performed at a Bruker DRX 9.4 Tesla Wide Bore NMR Spectrometer equipped with an actively shielded 40-mm gradient set (1 T/m maximum gradient strength). Cardiac function of mice was evaluated *in vivo* by acquisition of high resolution images using an ECG- and respiratory-triggered FLASH cine sequence. For metabolic studies, perchloric acid (PCA) extracts of wild-type (WT) and myo$^{-/-}$ hearts perfused for 20 min with 5 mM [1,6-$^{13}C_2$]glucose and 0.5 mM [U-^{13}C]palmitate were examined by ^{13}C MRS. Spectra of lyophilized extracts were recorded from a 5-mm ^{1}H/^{13}C dual probe. PET images were recorded after intravenous injection of 10 MBq [^{18}F]fluorodeoxyglucose (FDG) in 100 µl 0.9% saline using a submillimeter-resolution quad-HIDAC camera. Protein expression was analyzed by two dimensional gel electrophoresis (2D-PAGE) of whole heart extracts. Altered protein spots were identified by mass spectrometry (ESI-MS/MS).

Results and Discussion

Myocardial glucose utilization was non-invasively assessed using the radioactively labelled glucose analogon FDG in WT and myo$^{-/-}$ mice, which prior to PET analysis had been characterized by MRI to be functionally and morphologically normal. Myocardial FDG uptake was significantly enhanced in myo$^{-/-}$ as compared to WT mice (6.7±1.0 % of injected dose (ID) vs. 0.8±0.2 %ID, P<0.001) indicating increased cardiac metabolism of glucose. This metabolic switch was confirmed by ^{13}C NMR isotopomer studies of isolated hearts. Fig. 1 shows the isotopomer pattern of the glutamate carbon C4 of WT (bottom) and myo$^{-/-}$ (top) hearts perfused for 20 min with 5 mM [1,6-$^{13}C_2$]glucose and 0.5 mM [U-^{13}C]palmitate in the presence of 50 µU insulin. The sum of the resonances (S + D34) reports the amount of acetyl-CoA derived from [1,6-$^{13}C_2$]glucose (entry of [2-^{13}C]acetyl-CoA into the TCA cycle), and the sum of (D45 + Q) reflects the amount of acetyl-CoA derived from [U-^{13}C]palmitate (entry of [1,2-^{13}C]acetyl-CoA), irrespective of any cycling intermediate or pool sizes (3). Thus, in the example shown in Fig. 1, it is obvious that in Mb-deficient hearts the amount of carbons incorporated into glutamate which originate from glucose were increased over carbons originating from palmitate as compared to WT hearts. Quantitative analysis of the ^{13}C NMR spectra revealed that glucose utilization was significantly increased in myo$^{-/-}$ hearts (38±8% vs. 22±5% in WT), and concomitantly, palmitate utilization was significantly decreased in the Mb-deficient group (42±6% vs. 63±11% in WT, n=6, P<0.05). Proteom analysis revealed that key enzymes of mitochondrial ß-oxidation of fatty acids are drastically downregulated in Mb-deficient hearts, e.g. short-chain acyl-CoA dehydrogenase (-52%), isovaleryl-CoA dehydrogenase (-48%), enoyl-CoA hydratase (2 isoforms; -58%, -33%), while the glycolytic enzyme GAPDH was massively upregulated in Mb-deficient hearts (+474%). In summary, our data show that Mb aside of its role in tissue oxygen delivery plays a key role in muscle substrate selection. Since equimolar production of ATP from glucose consumes less oxygen than from fatty acids, this metabolic switch may be viewed as an additional adaptive mechanism in myo$^{-/-}$ hearts.

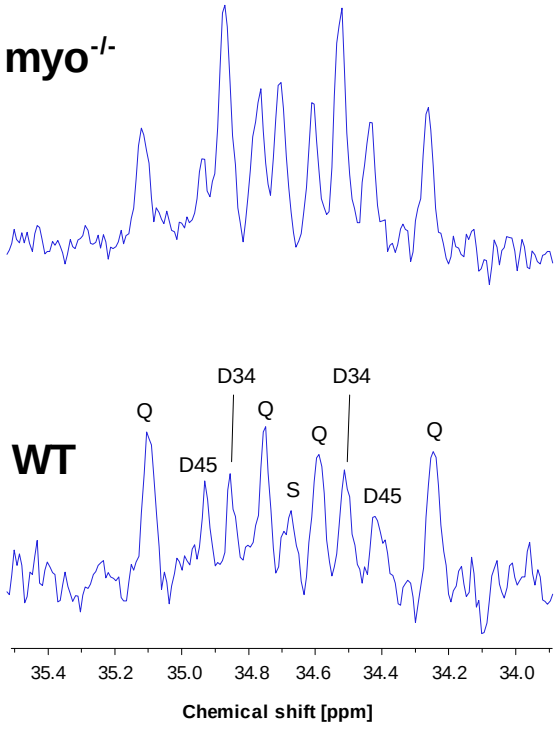

Figure 1: Sections of ^{13}C NMR spectra showing the glutamate C4 isotopomer pattern for WT and myo$^{-/-}$ PCA heart extracts. Abbreviations: D34, doublet due to J_{34} coupling (34 Hz); D45, doublet due to J_{45} coupling (51 Hz); Q, quartet (doublet of doublet) due to J_{345} coupling (J_{34} 34 Hz, J_{45} 51 Hz); S, singlet.

References

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