Regulation of murine myocardial energy metabolism during adrenergic stress studied by in vivo $^{31}$P NMR spectroscopy

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Naumova, A. V., R. G. Weiss, and V. P. Chacko. Regulation of murine myocardial energy metabolism during adrenergic stress studied by in vivo $^{31}$P NMR spectroscopy. *Am J Physiol Heart Circ Physiol* 285: H1976–H1979, 2003. First published July 24, 2003; 10.1152/ajpheart.00474.2003.—Image-guided, spatially localized $^{31}$P magnetic resonance spectroscopy (MRS) was used to study in vivo murine cardiac metabolism under resting and dobutamine-induced stress conditions. Intravenous dobutamine infusion (24 µg·min$^{-1}$·kg body wt$^{-1}$) increased the mean heart rate by $\sim$39% from 482 ± 46 per min at baseline to 669 ± 77 per min in adult mice. The myocardial phosphocreatine (PCr)-to-ATP (PCr/ATP) ratio remained unchanged at 2.1 ± 0.5 during dobutamine stress, compared with baseline conditions. Therefore, we conclude that a significant increase in heart rate does not result in a decline in the in vivo murine cardiac PCr/ATP ratio. These observations in very small mammals, viz., mice, at extremely high heart rates are consistent with studies in large animals demonstrating that global levels of high-energy phosphate metabolites do not regulate in vivo myocardial metabolism during physiologically relevant increases in cardiac work.

dobutamine stress; cardiac metabolism; magnetic resonance spectroscopy; PCr-to-ATP ratio

MYOCARDIAL ENERGY PRODUCTION is the highest per gram of any organ in the body and this fuels mechanical function (20). During increased contractile demand, such as during exercise or adrenergic stimulation, myocardial metabolic rates increase to supply the energetic needs (14, 15). The prime myocardial high-energy phosphates are ATP and phosphocreatine (PCr); these are reversibly converted by the creatine kinase (CK) reaction. Some studies in isolated cardiac mitochondria (7, 11, 27) indicate that increases in respiration are regulated by changes in high-energy phosphates, whereas most studies in intact large animals, including humans (3, 17, 23) demonstrate that global levels of high-energy phosphates do not regulate metabolism because they are unchanged during stress that increases heart rate. To the best of our knowledge, no in vivo studies of myocardial regulation have been conducted to date in very small animals, such as mice, at extremely fast heart rates.

Although high-energy phosphate levels can be determined by conventional biochemical techniques on digested tissue samples, $^{31}$P magnetic resonance (MR) spectroscopy (MRS) is the only noninvasive means for quantifying high-energy phosphates in the beating heart. $^{31}$P MRS has been used in isolated, perfused hearts (10) and has been combined with spatial localization techniques and $^1$H MR imaging (MRI) in intact large animals and humans (8, 12, 23, 28). Despite the importance of mice for transgenic manipulations, very little is understood about in vivo murine myocardial metabolic regulation. This, in part, is due to the very small size ($\sim$0.1 g) and very fast rates (500–600 per min) of normal mouse hearts. Studies of high-energy phosphate metabolism using $^{31}$P MRS have been reported in isolated mouse hearts (16, 19). Despite the ability to regulate substrate availability and myocardial perfusion in isolated heart preparations, such studies do not truly reflect the physiological conditions present in the intact, unperturbed in vivo setting. Recently, we implemented and validated image-guided, spatially localized $^{31}$P MRS techniques to study murine cardiac metabolism in vivo (6, 24). These studies demonstrated that the normal murine cardiac PCr/ATP ratio under baseline conditions is similar to that in larger species, including humans (1, 6, 17, 23).

The aim of the current studies was to utilize image-guided, spatially localized $^{31}$P MRS to study murine cardiac metabolism under resting and stress conditions in vivo to test the hypothesis that global levels of high-energy phosphates do not regulate myocardial respiration during substantial increases in cardiac demand in the normal mouse heart.

MATERIALS AND METHODS

All procedures and protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Johns Hopkins University. Detailed descriptions of the experimental protocols have been published elsewhere (6, 24). Briefly, adult mice (20–30 g) were lightly anesthetized with 1% isoflurane in oxygen (1 l/min) delivered through a nose
cone, and positioned on a flat Plexiglas platform with temperature control (37 ± 1°C). The tail vein was carefully catheterized for dobutamine delivery. Mice were placed in a custom-constructed 1H coil with the heart centered over the 31P coil (6, 24).

Experiments were performed on a NMR spectrometer (GE Omega) equipped with a 4.7 T/40 cm Oxford magnet and a 15 cm ID actively shielded Accustar gradient set (6, 24). Single-lead ECG was recorded from platinum electrodes attached to the mouse’s extremities for ECG-triggered MRI. Spin-echo transverse 1H MR images (11 ms echo time, 500 ms recycle time, 2 mm slice thickness, and 32 mm field of view) were used for localization and to confirm the position of the left ventricle over the center of the 31P MRS surface coil (OD 11 mm). Spatially localized 31P MR spectra were acquired after optimization of the magnetic field homogeneity with the use of the 1H coil to shim on a thick slice containing the heart. A one-dimensional chemical shift imaging sequence was used with 32 phase encode steps in the direction perpendicular to the plane of the coil. The time of the phase encode gradient was 0.5 ms, the field of view 32 mm, the recycle delay 1 s, and 64 averages were obtained per phase encode step. Adiabatic pulses with a flip angle of 45° were used for uniform excitation. Total acquisition time was ~34 min. With this protocol, well-resolved spectra from 1-mm slices parallel to the coil were obtained (6, 24). After completion of the first set of 31P spectra, dobutamine (Abbott Labs; Chicago, IL) was administered through the tail vein catheter (vide supra). Infusion was begun and gradually increased from 0 to 24 μg·min⁻¹·kg body wt⁻¹ in increments of 8 μg·min⁻¹·kg body wt⁻¹ at 3-min intervals. The dobutamine infusion was held constant at 24 μg·min⁻¹·kg body wt⁻¹, and another 31P MRS acquisition was started after the heart rate reached a new steady state. All mice awoke within ~1 min after completing the MRS examination.

1H MR images were analyzed with commercial software (NIH Image, version 1.52, Bethesda, MD), and 31P spectra were analyzed with a combination of custom (4) and proprietary software (NIH Image). The PCr-to-ATP (PCr/ATP) ratio was determined from the integrated peak areas of the creatine phosphate and β-PATP resonances from voxels centered on skeletal muscle or on cardiac muscle as identified from the high-resolution 1H MR images (Fig. 1), as described previously (6). Voxel shifting was performed when necessary to optimize slice alignment with cardiac structures and minimize skeletal muscle contamination of cardiac spectra (5). The PCr/ATP ratios were corrected for partial saturation effects using a factor of 1.2 determined in separate studies that included fully relaxed acquisitions (6, 24). Data during rest and dobutamine stress were compared using Student’s paired t-test and one-way ANOVA. Differences were considered statistically significant at P ≤ 0.05. All data are shown as means ± SD.

RESULTS AND DISCUSSION

Ten animals completed the entire 31P MRS dobutamine protocol and their data are presented here. The
mean baseline heart rate for all 10 animals was 482 ± 46/min (means ± SD). Intravenous infusion of dobutamine at the dose of 24 μg·min⁻¹·kg body wt⁻¹ significantly increased the mean heart rate by ~39% to 669 ± 77/min. The maximum β-adrenergic effect of dobutamine was detected ~12 min after initiation of dobutamine administration. Heart rates returned to baseline levels within a few minutes of stopping the dobutamine infusion.

A representative high-resolution short-axis ¹H MR anatomic image and spatially localized ³¹P MR spectra of myocardium, skeletal muscle, and the phenylphosphonic acid standard, acquired in the same examination, are shown in Fig. 1. The mean PCr/ATP ratios in intact heart and in skeletal muscle at baseline were 2.1 ± 0.5 and 3.0 ± 0.6, respectively (Fig. 2), after correction for partial saturation coefficient 1.2, as previously described (6, 24). These are similar to those reported before in normal mice (6). During intravenous administration of dobutamine the myocardial PCr/ATP ratio remained unchanged at 2.1 ± 0.5 (P > 0.05). As expected, the PCr/ATP ratio in skeletal muscle was also unchanged during dobutamine stimulation: 3.0 ± 0.6 and 3.2 ± 0.5, respectively (Fig. 2).

We studied cardiac high-energy phosphate levels in intact adult mice under baseline and dobutamine-stress conditions using image-guided, spatially localized ³¹P MRS and observed that the cardiac PCr/ATP ratio is unchanged even during a ~40% increase in heart rate in this small animal model. Our data are in agreement with studies in large species such as dogs, pigs, and humans, where increased heart rates did not result in a significant change in the cardiac PCr/ATP ratio (1, 3, 9, 23, 29). Therefore, we believe that increased mitochondrial respiration during adrenergic stimulation does not depend on changes in the relative global concentrations of myocardial phosphocreatine and ATP. This is consistent with the conclusion that global levels of high-energy phosphates do not regulate myocardial respiration, even in small animals, such as mice, with very rapid heart rates up to 750 beats/min. It is more likely that mitochondrial respiration is rather controlled by delivery of substrates, subsequent changes in the NADH/NAD redox, or mitochondrial calcium status (2, 3, 21, 22).

Mice are frequently used as models to study human physiology and disease because transgenic manipulations are more easily accomplished in that species. Despite the small size and rapid heart rates, our current studies demonstrate that it is feasible to perform studies of cardiac high-energy phosphate metabolism during sustained, reproducible dobutamine stress in intact mice with heart rates as high as 750 beats/min, with the use of image-guided, spatially localized ³¹P MRS.

Others (22) used MRI to demonstrate an increase in heart rate, cardiac output, and ejection fraction as well as a significant decrease of end-diastolic and end-systolic left ventricular volumes in normal mice after intraperitoneal injection of dobutamine. We studied three animals to separate functional testing with MRI during intravenous dobutamine infusion and also observed a similar increase in ejection fraction, cardiac output, and smaller end-systolic volumes during dobutamine stress (data not shown). Both intraperitoneal and intravenous dobutamine approaches result in increased heart rates, but the latter provides the opportunity for a more reproducible, graded, prolonged, and rapidly reversible method for adrenergic stimulation without uncertainties about dobutamine absorption.

Prior work using these spatial localization ³¹P MRS techniques in mice has shown that these measures are specific for myocardial metabolism (24) and largely uncontaminated by skeletal muscle (6). For example, in adult GLUT4 null mice a 60% increase in the PCr/ATP ratio is observed in cardiac but not in skeletal muscle slices using these noninvasive, image-guided ³¹P MRS localization techniques (24). Such observations of a comparable increase in cardiac but not skeletal PCr/ATP were separately confirmed in both isolated heart and in vivo open-chest studies of GLUT4 nulls (24). Those studies, with the GLUT4 null heart acting as an internal control or “phantom,” provide strong evidence for this study that those bioenergetic measures do indeed derive almost entirely from the intact murine heart.

In summary, it is now possible to study energetics at rest and during graded adrenergic stress in intact mice under physiological conditions. We report that a 40% increase in heart rate does not result in a significant change in the murine cardiac PCr/ATP ratio suggesting that global levels of high-energy phosphates and ADP do not regulate myocardial metabolism even in small mammals with extremely fast heart rates.

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DISCLOSURES

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