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Use of Magnetic Resonance Spectroscopy for In Vivo Evaluation of High-Energy Phosphate Metabolism in Normal and Abnormal Myocardium

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ABSTRACT

^{31}P- and ^{1}H-nuclear magnetic resonance spectroscopy (MRS) are powerful tools for studying myocardial energy metabolism. The purpose of this review is to illustrate how these MRS techniques can be used to study complex bioenergetic issues in normal and abnormal in vivo myocardium. The results provide insight into the energetic alterations present in remodeled and hypertrophied myocardium. A detailed understanding of energy metabolism in normal and abnormal myocardium may point the way to improved preventive, diagnostic, and therapeutic modalities for left ventricular dysfunction.
KEY WORDS: *Deoxymyoglobin; High-energy phosphates; Left ventricular hypertrophy; Left ventricular remodeling; Myocardial ischemia.*

INTRODUCTION

Recent developments have established magnetic resonance spectroscopy (MRS) as a powerful technique for study of the energy metabolism of the heart. Using ^{31}P-MRS, the myocardial high-energy phosphate (HEP) compounds ATP, creatine phosphate (CP), and inorganic phosphate (P_i) can be nondestructively detected in human (1–5) and animal (6–19) myocardium under in vivo conditions. In additon, cytosolic pH can be derived from the P_i chemical shift and cytosolic free ADP content can be calculated from the creatine kinase equilibrium relationship (20). Because of the central role of these compounds in bioenergetics, ^{31}P-MRS can provide new and unique insights into myocardial energy metabolism in both normal and pathological states. Furthermore, it is also possible to assess the state of intracellular oxygenation using ^{1}H-MRS to determine the deoxymyoglobin content of in

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vivo myocardium (21). These measurements of oxygenation are of fundamental importance for interpretation of HEP changes that often occur during physiological or pathological alterations of myocardial performance.

Until relatively recently, most myocardial MRS studies were performed with isolated perfused hearts. This model system by necessity treats the heart as a biochemically homogeneous system operating independently of extracardiac physiological regulatory mechanisms. However, in the in vivo myocardium, myocardial energy metabolism, perfusion, and contractile function are known to be regionally and transmurally nonuniform, and each of these variables can be affected by changes in systemic hemodynamics, exogenous carbon substrate availability, and oxygen availability. Regional nonuniformity is amplified during and subsequent to an ischemic insult, especially when a perfusion deficit is induced by a coronary stenosis (7,9,14). Studies of in vivo cardiac muscle using spatially localized ^{31}P-MRS have permitted the examination of transmural metabolic characteristics (Fig. 1 and Appendix). In these studies, measurements of the transmural myocardial perfusion pattern (microsphere technique) and transmural systolic function (ultrasonic crystals) obtained concomitantly with MRS data have allowed evaluation of the interactions between these variables across the left ventricular (LV) wall in open chest dogs (7,14).

A number of problems are best studied with MRS techniques. For example, myocardial ischemia is by definition a state in which the oxygenated blood supply is insufficient to meet myocardial metabolic demands. In general, the presence of myocardial ischemia is based on evidence of decreased blood flow, decreased contractile function, alterations of the lactate uptake/efflux status, or combinations of these indices (14). ^{31}P-MRS studies demonstrate a decrease in the CP/ATP ratio, reflecting an increase of myocardial free ADP as ischemia slows the rate of ATP synthesis below the rate of ATP consumption by the contractile apparatus and membrane pumps. The P_i liberated as ATP hydrolysis exceeds the rate of ATP synthesis appears as a discrete resonance in the ^{31}P spectra. In principle, a borderline ischemic state could be detected by ^{31}P-MRS manifested only by changes in the characteristics of regulation of oxidative phosphorylation without myocardial contractile alterations (14). Because these HEP changes during ischemia are the result of insufficient oxygen availability to support mitochondrial respiration, ^{1}H-MRS studies can document the presence of deoxymyoglobin, indicating reduced intracellular oxygen availability.

A decreased CP/ATP ratio (as an indication of an increase of the myocardial free ADP level) has been found in patients with heart disease (1,5) and in large or small animal models of LV hypertrophy secondary to pressure overload (10,12,13,17), volume overload (19), and post-

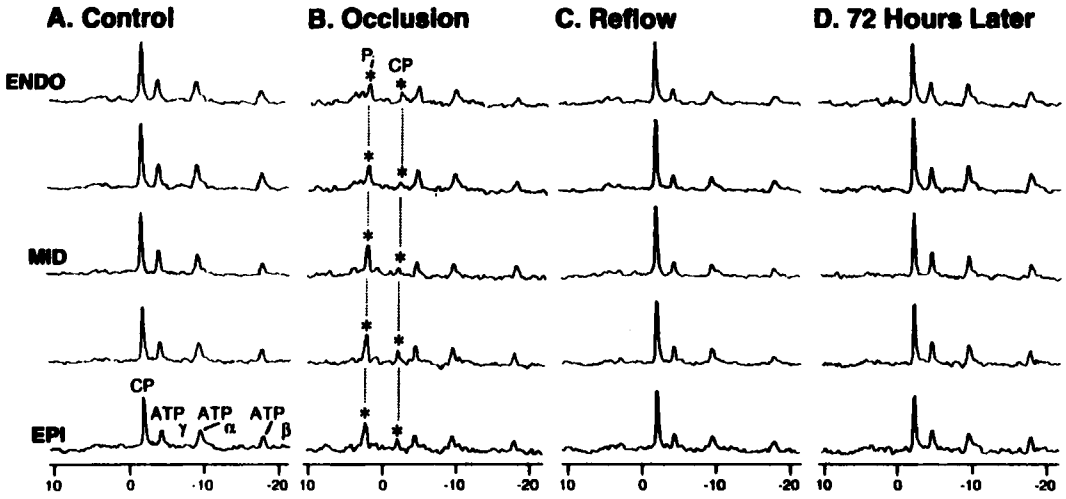

Figure 1. ^{31}P-MRS sets in five transmural layers from epicardium (EPI) to endocardium (ENDO) from a normal closed chest dog in which myocardial stunning was produced with three successive 10-min coronary occlusions. Each transmural data set consists of a stack of five spectra corresponding to voxels spanning the LV wall from epicardium to endocardium. The MR localization technique is described in the Appendix. Spectra shown are during basal conditions (A), during a 10-min total occlusion of left anterior descending coronary artery that resulted in myocardial stunning (B), after 20 min of reflow (C), and after 72 hr of reflow (D). Under basal conditions, the CP/ATP ratio is highest in epicardium. A decrease of CP and ATP and an increase of P_i are evident during occlusion; CP recovered rapidly during reflow, whereas ATP recovered only gradually over the next 3 days.

infarct LV remodeling (LVR) (15,18,22). These HEP abnormalities could be the result of primary alterations of the characteristics of oxidative phosphorylation, limitations of oxygen and/or substrate delivery, or abnormalities of intermediary metabolism. It is uncertain whether ATP synthetic capacity is limited by any of the aforementioned factors or whether these abnormalities can contribute to the progression of LV dilation and myocardial dysfunction. Alterations in calcium dynamics and of both contractile and regulatory proteins of the contractile apparatus have been reported in hypertrophied and failing hearts (23–29), suggesting that myocardial dysfunction may be, at least in part, a consequence of abnormalities of the contractile apparatus and support systems. The relative contributions of abnormal myocardial geometry, primary abnormalities of contractile function, and ATP synthetic or transport limitations to myocardial dysfunction are not known at present.

This review is presented with the intent of illustrating the power of MRS to examine biochemical and physiological questions in in vivo heart. Representative studies of postinfarction LVR, pressure overload LV concentric hypertrophy, and volume overload eccentric LV hypertrophy are presented. The discussion is, in the main, focused on recent work from this laboratory and is not intended to be an exhaustive review of either the cardiac applications of MRS or the specific topics addressed. Studies of the regulation of oxidative phosphorylation in the perfused heart and the open chest intact heart have been reviewed elsewhere (19,30).

MYOCARDIAL HEP LEVELS IN HEARTS WITH POSTINFARCTION LVR

Acute myocardial infarction results in remodeling of the noninfarcted region of the left ventricle, which can ultimately lead to the development of congestive heart failure (CHF) (18,22,31,32). Although HEP abnormalities have been observed in remodeled myocardium (15, 18,22,32), it is unclear whether they are caused by perfusion abnormalities or altered intermediary metabolism or even whether these bioenergetic abnormalities can contribute to the development of CHF. We used a porcine model of postinfarction LVR to examine the relationships between the severity of LV dysfunction and abnormalities of myocardial HEPs (18). Proximal left circumflex coronary artery occlusion resulted in infarct of 20–25% of the LV myocardium. Over the next several months, approximately one third of the animals developed clinical CHF, whereas the remaining animals had LV dilation (LVR) without CHF. Compared with normal animals, LV ejection fraction determined with magnetic resonance imaging decreased significantly from 56 ± 5.6% to 35 ± 2.3% in LVR group and 24 ± 2.8% in CHF group.

As shown in Table 1, CP/ATP ratios determined in LV myocardium remote from the infarct using spatially localized ^{31}P-MRS were significantly decreased in the subendocardium of hearts with compensated LVR. The decrease of the CP/ATP ratio was much more marked in hearts that developed CHF and occurred in all transmural myocardial layers. CP/ATP ratios were significantly lower in animals with clinical signs of CHF than in animals with compensated LVR. Furthermore, the decreases of CP/ATP and the severity of LV remodeling were correlated with the degree of myocardial damage estimated from the ratio of LV scar surface area to total LV surface area. Figure 2 illustrates typical spectra obtained from a normal heart (A), a heart with LVR (B), and a heart with LVR plus CHF (C). Calculated myocardial free ADP levels were increased only in CHF hearts. Figure 3 illustrates transmural sets of ^{31}P-MRS acquired from a normal heart under basal conditions (A) and during intracoronary in-

Table 1

Anatomic and Magnetic Resonance Imaging Results

	n	LVR/BW (g/kg)	EF (%)	CP/ATP Ratio		
				Subepicardium	Midwall	Subendocardium
Normal	10	3.03 ± 0.12	56 ± 5.6	2.10 ± 0.10	2.06 ± 0.16	1.92 ± 0.12
LVR	12	3.65 ± 0.25*	35 ± 2.3*	1.99 ± 0.13	1.80 ± 0.14	1.57 ± 0.15*
LVR + CHF	6	4.99 ± 0.42*†	24 ± 2.8*†	1.41 ± 0.14*	1.33 ± 0.15*†	1.25 ± 0.15*†

Values are means ± SEM.
*$p < 0.05$ vs. respective Normal.
†$p < 0.05$ vs. LVR.
EF, ejection fraction; BW, body weight.

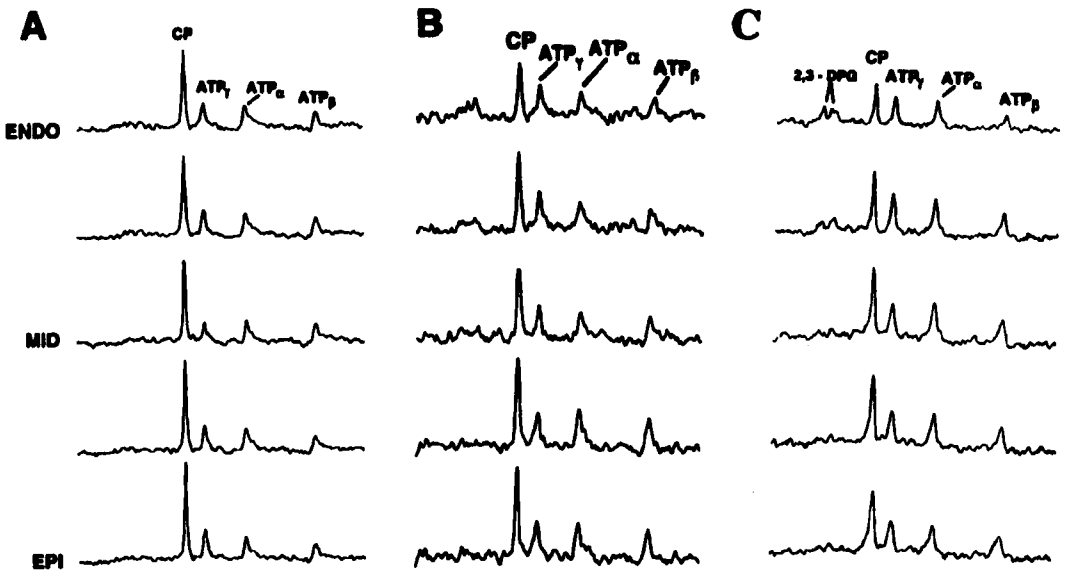

Figure 2. ^{31}P-MRS from five transmural layers across the LV wall of a normal heart (A), a heart with compensated LVR (B), and a heart with CHF (C) under basal conditions. Spectra were scaled to optimize visualization of the resonances, so that only the CP/ATP ratio can be compared. The CP/ATP ratio was reduced in endocardium in LVR hearts and in all transmural layers in the heart with CHF. ENDO, endocardium; MID, midwall; EPI, epicardium; 2,3-DPG, 2,3-diphosphoglycerate.

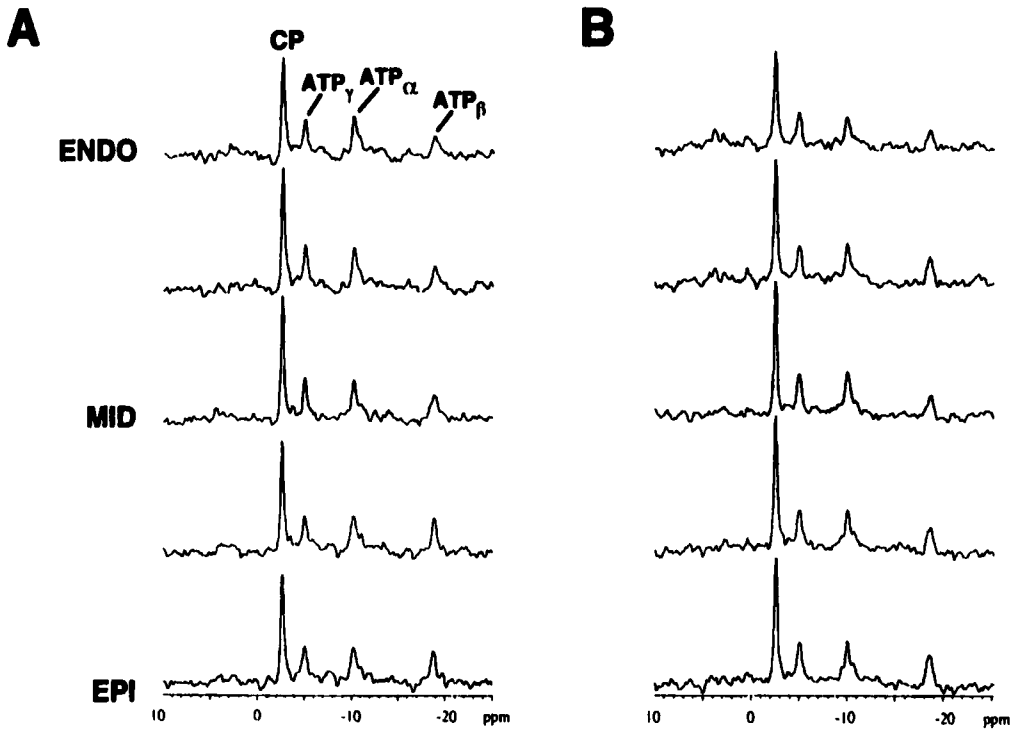

Figure 3. Transmurally differentiated ^{31}P-MRS under basal conditions (A) and during intracoronary pyruvate infusion (B) in a normal heart. ENDO, endocardium; MID, midwall; EPI, epicardium.

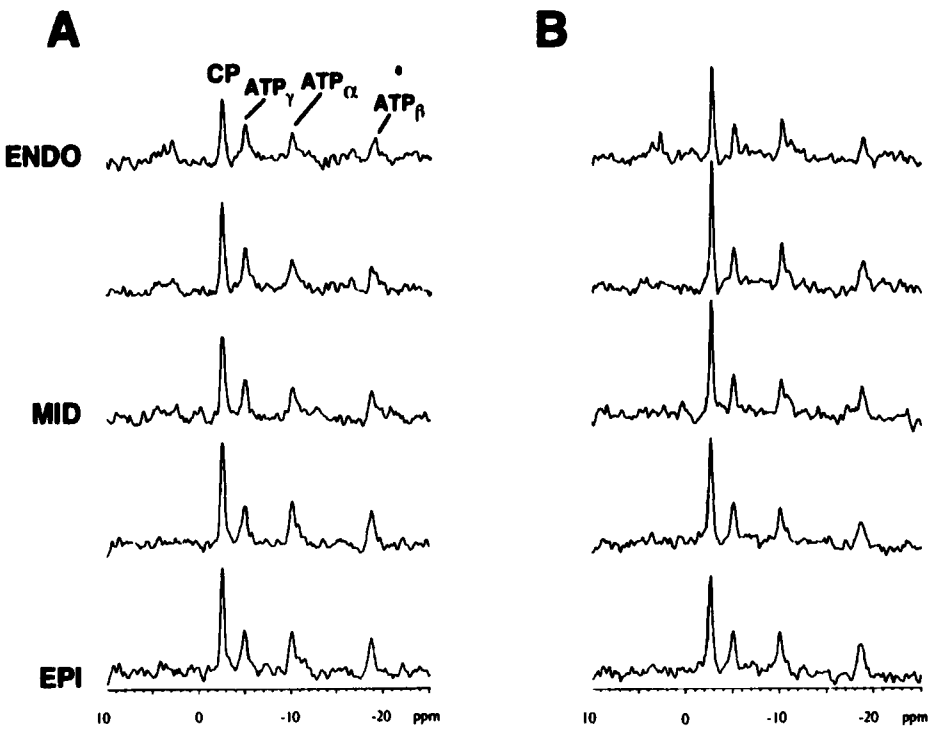

Figure 4. Transmurally differentiated ^{31}P-NMR spectra under basal conditions (A) and during intracoronary pyruvate infusion (B) in a heart with compensated postinfarction LV remodeling. During basal conditions, the CP/ATP ratio in the subendocardial layer is decreased as compared with normal (See Fig. 3A). Intracoronary infusion of pyruvate corrected the depressed subendocardial CP/ATP ratio in the remodeled heart. ENDO, endocardium; MID, midwall; EPI, epicardium.

fusion of pyruvate (B). Corresponding spectra from an LVR heart are shown in Figure 4.

Pyruvate infusion resulted in a significant increase of the subendocardial CP/ATP in LVR hearts but not in normal hearts. Interestingly, pyruvate infusion did not improve the depressed CP/ATP ratio in animals with CHF. To determine whether the HEP alterations in the postinfarct hearts were the result of myocardial hypoperfusion, adenosine was administered to cause an increase of coronary blood flow. Adenosine infusion caused no improvement of the abnormal HEP, indicating that these alterations are not the result of persistent myocardial hypoperfusion. Thus, the HEP abnormalities in postinfarct remodeled myocardium are related to the severity of LV dysfunction that, in turn, depends on the size of the initiating infarct (18). The decreased CP/ATP ratio in LVR (but not CHF hearts) can be partially explained by abnormalities of carbohydrate metabolism, because intracoronary infusion of pyruvate tended to normalize the depressed CP/ATP ratio. However, the data do not support the concept that persistent underperfusion of the myocardium is the cause of these HEP abnormalities.

LVR SECONDARY TO DISCRETE MYOCARDIAL INJURY WITHOUT INTERRUPTION OF CORONARY BLOOD FLOW

LVR secondary to discrete necrosis without coronary occlusion can be produced by transmyocardial direct current electrical shock (15). After the discrete injury, the LV undergoes progressive dilatation. At 1 yr after creating a focal LV scar in dogs, LV mass and end-diastolic volume were increased by 33% and 26%, respectively. Under basal conditions, the subendocardial CP/ATP ratio was significantly lower in remodeled as compared with normal hearts; this alteration was inversely correlated with both the degree of LV dilatation and the degree of hypertrophy. Unlike normal hearts, in remodeled myocardium, pacing stress caused a significant increase in LV end-diastolic pressure that was accompanied by a further decrease in the subendocardial CP/ATP ratio. This decrease in the subendocardial CP/ATP ratio was correlated with a decrease of an endocardium-to-epicardium blood flow ratio during pacing. Thus, both in this model

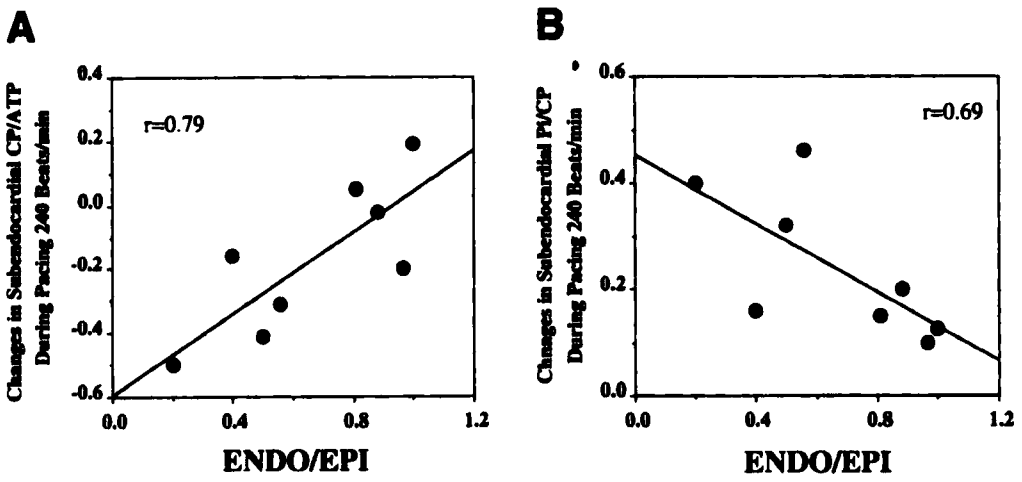

Figure 5. Transmural blood flow distribution, expressed as the subendocardial blood flow/subepicardial blood flow (ENDO/EPI) ratio, plotted against changes in subendocardial CP/ATP ratio (A) and $\Delta P_i/CP$ (B) in eight dogs with LVR during pacing at 240 beats/min.

of discrete myocardial injury without interruption of coronary flow and in the case of remodeling secondary to infarct resulting from coronary artery occlusion, the alterations in myocardial HEP levels are correlated with the extent of LVR. Even in the absence of coronary occlusion, rapid atrial pacing caused additional reductions of subendocardial myocardial HEP levels that appeared to be related to redistribution of blood flow away from the subendocardium (Fig. 5) (15).

MYOCARDIAL OXYGENATION IN HEARTS WITH POSTINFARCTION LVR

In the failing heart, energy demand is increased to support the increased systolic wall stress of the dilated left ventricle, whereas oxygen delivery may be limited by an impaired coronary flow reserve. These considerations have led to the suggestion that an imbalance of the energy delivery–demand relationship might contribute to impaired contractile performance in the failing heart (31). In experimental models of postinfarction LVR, decreases of CP, ATP, the CP/ATP ratio, and total creatine occur, and these changes are most prominent in animals that develop CHF. Thermodynamic and kinetic models emphasize roles for ADP, P_i, mitochondrial NADH (which reflects delivery of carbon substrate and the integrity of the tricarboxylic acid cycle), and O_2 in regulation of myocardial oxidative phosphorylation (30,33,34). Thus, the HEP abnormalities in the remodeled hearts could be caused by limitations of carbon substrate or oxygen availability, by primary alterations in the process of oxidative phosphorylation, or by oxygen limitation. Determination of intracellular O_2 is necessary to determine whether oxygen limitation contributes to the HEP and contractile abnormalities observed in remodeled myocardium.

Kreutzer and Jue (35,36) described the use of ^{1}H-MRS measurements of myocardial deoxymyoglobin (Mb-δ) to assess mitochondrial oxygen availability in isolated perfused rodent hearts. We adapted this methodology for study of the in vivo heart. In a recent ^{1}H-MRS study in normal dogs in vivo, we were unable to detect myoglobin desaturation under basal conditions (21). However, graded coronary artery stenoses caused increases of the Mb-δ signal that were linearly correlated with the decreases of myocardial blood flow. These data demonstrate that under normal blood flow conditions, myocardial intracellular Po_2 is relatively high (as indicated by the absence of detectable Mb-δ) and therefore nonlimiting to oxidative phosphorylation. The appearance of an Mb-δ resonance when myocardial blood flow was reduced below normal levels demonstrated that the ^{1}H-MRS method is capable of detecting Mb-δ in vivo with an excellent degree of sensitivity.

This methodology was used to test the hypothesis that limited myocyte oxygen availability is the basis for the HEP abnormalities in postinfarct remodeled myocardium (32).^{31}P- and ^{1}H-MRS were used to evaluate HEP levels and Mb-δ in myocardium remote from the infarct in

swine 2 months after occlusion of the left circumflex coronary artery. Nine of 23 LVR animals had CHF. As previously described, basal CP/ATP tended to be decreased in postinfarct hearts, and this was significant in animals with CHF. Infusion of dobutamine (20 μg/kg/min IV) caused doubling of the rate-pressure product in both normal and LVR hearts and resulted in comparable significant further decreases of CP/ATP in both groups. These decreases in CP/ATP were not associated with detectable MB-δ, although coronary occlusion (performed as a control) always generated a large Mb-δ resonance. In CHF hearts, rate-pressure product increased only 40% in response to dobutamine; this attenuated response also was not associated with detectable Mb-δ. Thus, the decrease of CP/ATP during dobutamine infusion was not the result of insufficient oxygen available to the myocardium. Furthermore, even in CHF hearts, the low basal CP/ATP and the attenuated response to dobutamine occurred in the absence of myocardial hypoxia, indicating that the HEP and contractile abnormalities were not the result of limited oxygen availability.

LV HYPERTROPHY SECONDARY TO PRESSURE OVERLOAD

Hearts with severe pressure overload LV hypertrophy demonstrate prominent HEP abnormalities even during basal conditions (2,8). We produced severe LV hypertrophy by banding the ascending aorta in dogs 8 weeks of age. As the animals undergo normal body growth, the area of constriction remains fixed, resulting in a progressively increasing degree of stenosis, so that by adulthood a near doubling of LV mass is observed. Transmural ^{31}P-MRS studies demonstrated HEP abnormalities during the basal state; ATP was decreased by 42%, CP was decreased by 58%, and the CP/ATP ratio by 32% compared with the respective values from a normal control group. The decrease of the CP/ATP ratio was strongly correlated with the severity of hypertrophy. Consequently, calculated free ADP levels were increased in direct proportion to the severity of LV hypertrophy. Hyperperfusion produced by coronary vasodilation with adenosine did not improve the HEP levels in the LV hypertrophy hearts, indicating that the decreased CP/ATP ratios were not caused by persistent myocardial underperfusion. When myocardial metabolic demands were increased by atrial pacing, LV hypertrophy hearts showed further depletion of HEP levels most prominent in the subendocardium (2). This was associated with a redistribution of blood flow away from subendocardium during pacing. These pacing-induced abnormalities were not observed in the normal hearts. Thus, the decreased HEP levels and decreased CP/ATP ratios during basal conditions in severely hypertrophied pressure-overloaded hearts are not the result of persistent myocardial underperfusion. However, during tachycardia, the hypertrophied hearts are vulnerable to subendocardial hypoperfusion that results in further loss of HEP due to ischemia.

LV HYPERTROPHY SECONDARY TO VOLUME OVERLOAD

Chronic mitral regurgitation can result in progressive LV dilation with eccentric hypertrophy and eventual dysfunction. We studied myocardial HEP content and contractile function in hearts with chronic volume overload secondary to mitral regurgitation 1 yr after disruption of the chordal apparatus (19). Mitral regurgitation caused a 65% increase in LV volume with a 30% increase in LV mass. In animals with mitral regurgitation, LV function was normal at rest, but mild dysfunction was apparent during treadmill exercise. Myocardial CP/ATP ratios were significantly lower in each layer across the LV wall compared with normal. Myocardial blood flow and the coronary flow reserve were normal in hearts with mitral regurgitation. Moreover, myocardial hyperperfusion did not correct the abnormal CP/ATP ratios. Unlike LV hypertrophy secondary to pressure overload, the hearts with volume overload hypertrophy did not display further changes in CP/ATP or P_i/CP ratios during rapid pacing or during dobutamine infusion. The findings demonstrate that in volume-overloaded hearts with eccentric hypertrophy, alterations in myocardial HEP levels are not associated with abnormal mechanical performance at rest. However, it is possible that abnormalities in ATP synthesis could contribute to a decreased contractile reserve during very high workstates.

SUMMARY

The major purpose of this review was to demonstrate that ^{31}P-MRS techniques can be used to study complex bioenergetic issues in the in vivo myocardium in ways not previously feasible. It is likely that future studies at higher field strengths using extrathoracic surface coils in intact sedated animals and in human subjects will allow investigators to more clearly define normal and abnormal myocardial bioenergetic processes and their regulation in the intact organism. It is anticipated that future studies will answer the persisting question of whether limitations

of ATP synthetic or transport capacities contribute to the pathogenesis of LVR or failure.

APPENDIX

^{31}P-MRS with transmural differentiations was performed using a 4.7-T Oxford magnet interfaced to a SIS Co. console. A surface coil constructed of a 28-mm diameter single circular loop of copper wire incorporating a single 33 pF capacitor was placed on the epicardial surface of the left ventricle. A polyethylene capillary filled with 15 μl of 3 M phosphonoacidic acid was placed at the center of the coil to facilitate calibration and to serve as an epicardial marker. Spatially localized ^{31}P-MRS was performed using the RAPP-ISIS technique* (7,10, 15,16,19,3,28). This is the phase-modulated analog of the FLAX-ISIS method† described elsewhere in detail (37,38). Detailed data with regard to voxel profiles, voxel volume, and documentation of the accuracy of localization achieved in phantom studies and in the intact in vivo heart have been previously published (37,38).

In this application of RAPP-ISIS, signal origin was restricted to a 18 × 18 mm column perpendicular to the surface coil plane and hence the LV wall; localization along the column (across the LV wall) was achieved with B_1 based phase encoding where the number of transients accumulated for each phase-encode step was weighted according to a nine-term Fourier series window, as previously described (10,37,38). The phase-encoded data were used to generate a voxel or a "window" that can be shifted arbitrarily by postdata acquisition processing along the phase-encode direction. In these studies, voxels were generated at five distances or "depths" from the surface coil centered about 45-, 60-, 90-, 120-, and 135-degree phase angles. The position of the voxels relative to the coil was set according to the B_1 strength at the coil center that was experimentally determined in each case by measuring the 90-degree pulse length for a reference contained in a capillary placed in the center of the surface coil. The 18 × 18 mm column was defined using sech/tanh modulated, 1.5- to 2-msec-long, adiabatic inversion pulses and 2.5- to 3.0-G/em B_0 gradients. The adiabatic excitation pulse that follows the adiabatic inversion pulse in RAPP-ISIS was based on optimized functions and was typically 1 msec in length (38).

* RAPP-ISIS technique: The rotating-frame experiment using adiabatic plane-rotation pulses for phase modulation (RAPP)–imaging-selected in vivo spectroscopy (ISIS).

† FLAX-ISIS method: Fourier series window longitudinally modulated, using adiabatic excitation ISIS technique.

Complete transmural data sets were obtained in 10-min time blocks using a repetition time of 6–7 sec that allowed essentially complete relaxation of ATP and P_i resonance and ~90% relaxation of the CP resonance; the CP data were corrected for the saturation effect using a fully relaxed whole wall spectra acquired at the beginning of each study. MRS data acquisition was gated to the cardiac and respiratory cycles as previously described by using the cardiac cycle as the master clock that drives both the respirator and the spectrometer (37).

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