MR imaging measurement of compartmental water diffusion in perfused heart slices

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MR imaging measurement of compartmental water diffusion in perfused heart slices. Am J Physiol Heart Circ Physiol 281: H1280–H1285, 2001.—Myocardial tissue slices were isolated from the left ventricular free wall (7 slices) and left ventricular papillary muscle (3 slices) of New Zealand White male rabbits (n = 4) and were subsequently superfused with a modified St. Thomas’ Hospital cardioplegic solution at 19°C. The diffusion-weighted images were obtained with a 600-MHz nuclear magnetic resonance spectrometer using diffusion gradient b-values that ranged from 166 to 6,408 s/mm²; the apparent diffusion coefficient of water in the tissues was subsequently calculated. All of the tissue samples that were studied exhibited nonmonoexponential diffusion. Data from seven slices were mathematically fitted by a biexponential expression with a fast diffusion component of 0.72 ± 0.07 × 10⁻³ mm²/s, and a slow diffusion component of 0.060 ± 0.033 × 10⁻³ mm²/s. The fast component dominated the calculated apparent diffusion coefficient of the tissue, composed of 82 ± 3% of the overall diffusion-dependent signal decay. Thus myocardial tissue exhibits characteristics consistent with multiple compartments of diffusion. This work has important implications for myocardial diffusion tensor imaging, as well as the changes in diffusion that have been reported following myocardial ischemia.

heart; diffusion; nuclear magnetic resonance; magnetic resonance imaging

RECENT REPORTS (10, 12, 18) have used myocardial diffusion imaging to provide information regarding the microstructural organization of the heart, and uncovered alterations in the apparent diffusion coefficient (ADC) of water that occur during myocardial ischemia (13). Moreover, the prior demonstration (9, 17) that myocardial diffusion imaging can be performed in vivo has dramatic implications for clinical applications. Findings from rat brain slices (6), the in vivo rat brain (15), and the human brain (14) suggest that the ADC of water may be biexponential in these tissues, raising the possibility that multiple water compartments are being observed. A recent report has shown the feasibility of performing multiexponential diffusion imaging in a clinical magnetic resonance (MR) scanner (14), providing evidence that multicompartmental diffusion imaging may eventually have direct clinical application. The current study was performed to determine if nonmonoexponential water diffusion exists in the heart as previously reported for neuronal tissue.

Myocardial fiber orientation plays a significant role in the patterns of electrical depolarization, the development of myocardial stress and strain, and indirectly has a role in local myocardial perfusion and oxygen consumption. Hsu et al. (13) recently reported the first quantitative correlation of histologically determined myocardial fiber orientations in the excised right ventricle with the principal eigenvector of the diffusion tensor. Scollan et al. (18) subsequently reported a correlation between the anatomic microstructure of the intact perfused rabbit heart (myocardial fiber angle orientation and laminar sheet structure) with the principal and tertiary eigenvectors of diffusion. This work provided direct evidence that diffusion tensor imaging could furnish information regarding anatomic structure that was previously available through laborious histological examination on fixed tissue.

In addition, myocardial fibers are altered in a number of disease states, such as ischemia (2, 16), diabetes (21, 22), hypertensive cardiomyopathy (1), and hypertrophy (20). Recent advances have permitted the clinical application of diffusion-weighted imaging techniques to the study of the heart, where it may provide new information regarding the efficacy of treatment or enhanced detection of pathophysiological alterations. Hsu et al. (13) reported decreased ADC in the myocardium following acute regional ischemia. In contrast to the temporal characteristics of diffusion changes that have been observed in the brain after ischemia, changes in the ADC of water in the ischemic heart are delayed, and changes in T₂-weighted images preceed observed changes in ADC images (13). The decrease...
in ADC reported in both brain and heart has been attributed to cytosolic swelling due to a loss of ionic homeostasis, though other mechanisms may contribute.

Previous studies (9, 10, 12, 13, 17, 18) in the heart were limited by the strength of the diffusion gradient strengths and subsequently small \( b \)-values. As a result, the tensors that were calculated (12, 17, 18) were biased toward the fast component of diffusion (assuming two compartments); the slow component (if present) may not be apparent at \( b \)-values <1,000 s/mm\(^2\). The slow component might have a substantial contribution to the overall tensor calculations, depending on relative sizes of the compartments corresponding to the fast and slow components. Moreover, the decreased diffusion that has been observed in the heart following ischemia (13) could be a result of alterations in the relative size of these compartments rather than a uniform reduction of water ADC in the affected region. The goal of the present study was thus to determine if the ADC in heart tissue is mono- or multiexponential and discuss the impact of this on the measurement of the ADC and the diffusion tensor in the heart.

**MATERIALS AND METHODS**

*Sample preparation.* New Zealand White male rabbits (1.5 kg) were euthanized by an anesthetic overdose (44 mg/kg im ketamine and 15 mg/kg im xylazine), followed by rapid exsanguination. Hearts were isolated and placed in ice-cold (4°C) modified St. Thomas’ cardioplegic solution that had been equilibrated with 95% O\(_2\)-5% CO\(_2\). The perfusate contained (in mM) 110 NaCl, 16 KCl, 16 MgCl\(_2\), and 10 NaHCO\(_3\). Dextrose (5 mM) and adenosine (1 mM) were added to improve long-term viability and the pH was adjusted to 7.4. Hearts were perfused for \( \sim 10 \) min (at 19°C) to remove blood from the vascular compartment and to arrest the heart. The hearts were then removed, placed into the ice-cold buffer, and sectioned perpendicular to the long axis of the left ventricle (see Fig. 1). A section of the left ventricular free wall was isolated and coarse sectioned into a block of tissue \( \sim 5 \) mm\(^2\) x 3 mm thickness. This was subsequently finely sectioned into several slices spanning the transmural direction of the left ventricular free wall (400–700 \( \mu \)m thickness). In three of the hearts, the papillary muscle was also isolated and sectioned perpendicular to the long axis of the ventricle (2 mm diameter, 500–900 \( \mu \)m thickness). The tissue slices were placed into a tissue perfusion chamber, as described previously (3), which was inserted into a 10 mm nuclear MR (NMR) tube, the temperature maintained at 19°C, and the perfusion rate adjusted to 1 ml/min. The flow was stopped during the diffusion imaging sequences to minimize motion artifacts.

*NMR imaging.* Images were acquired on a 600-MHz spectrometer (Varian). A pulsed gradient spin-echo sequence was used with a spatial resolution of 117 \times 156 \times 300 \( \mu \)m, repetition time = 2 s, echo time = 30 ms, 128 \times 64, number of averages = 2, for a total imaging time of 4.3 min per image. Diffusion weighting was achieved by using two 3.5-ms square gradient pulses separated by 12 ms and applied in the slice-select direction. The amplitude of the diffusion gradients ranged from 2 to 75 G/cm. This resulted in the following range of \( b \)-values: 166, 220, 321, 490, 918, 1,734, 3,602, and 6,408 s/mm\(^2\). A biexponential function was fitted to the data as previously described (6)

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SI = M(0) \cdot [f_1 \cdot e^{-bD_1} + (1 - f_1) \cdot e^{-bD_2}]
\]

where SI is the signal intensity, \( e \) is the exponent, \( M(0) \) is the magnetization in the absence of diffusion gradients, \( f_1 \) is the signal fraction characterizing the slow diffusion component.
$D_2$ is the fast diffusion coefficient, and $D_1$ is the slow diffusion coefficient.

Statistical analysis. Data were analyzed by defining regions of interest that corresponded to the tissue, the surrounding superfusate, and the area outside the coil (for determination of signal-to-noise ratio). Statistical analysis was performed using nonlinear curve fitting and the $F$-test ratio (5) with statistical significance established at $P < 0.05$.

RESULTS

Ten slices were obtained from four hearts. Seven of the slices were from the LV free wall, and three were samples of papillary muscle from different hearts. Figure 2 shows diffusion-weighted images from one slice. At the higher diffusion weighting (Fig. 2B), the signal from the faster diffusing-surrounding perfusate is suppressed, leaving signal only from the heart slice itself. At these spatial resolutions, the image is unremarkable and no internal structure is discerned with the diffusion gradient in the $z$-orientation, as expected. All tissue samples exhibited a similar biexponential decline in signal intensity when plotted as a function of diffusion gradient $b$-values. Preliminary analysis of the ADC based on anatomic location showed no differences between the tissue obtained from the papillary muscle and the LV free wall; as a result, all tissue samples have been grouped for clarity (see Fig. 3). Of the ten slices that we studied, three suffered from significant image artifacts and their data was discarded, and thus seven slices were analyzed. The results indicate the calculated ADC of the fast diffusion coefficient corresponds to $0.72 \pm 0.07 \times 10^{-3}$ mm$^2$/s, and is composed of $82 \pm 3\%$ of the total diffusion constant. The slow diffusion coefficient was estimated at $0.060 \pm 0.033 \times 10^{-3}$ mm$^2$/s. The biexponential model better described the data than a monoexponential ($F$-test, $P < 0.0005$).

DISCUSSION

These data demonstrate for the first time that diffusion in myocardial tissue is nonmonoexponential, and can be fitted by a biexponential expression as previously reported for brain tissue (6). This has been speculated to arise from water compartmentation, which may possibly arise from the intra- and extracellular compartments. Evidence for the source of the two compartments remains circumstantial although experiments performed with ouabain (6) and $\text{N}$-methyl-$\text{D}$-aspartate (NMDA) (7) in brain tissue slices have indicated that the increased intracellular volume that

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Fig. 2. Representative images and illustration of myocardial fiber angle orientation. A: light diffusion-weighted image of the heart slice surrounded by perfusate, $b = 166$ s/mm$^2$. B: heavy diffusion-weighted image of the same slice (different windowing), $b = 1,734$ s/mm$^2$. Perfusate signal has dropped below noise level. Both images show relatively homogeneous tissue contrast. C: myocardial fiber angle orientation relative to the images; progression from epicardial to endocardial regions. Diffusion gradient is oriented along the $z$-axis.
accompanies ouabain or NMDA administration corresponded to similar changes in the compartment size for the slow component of diffusion. Further experiments are required to elucidate the origin of this compartmentation.

The ADCs that were measured in heart tissue slices (0.72 ± 0.07 × 10^{-3} mm^2/s for the fast component, and 0.060 ± 0.032 × 10^{-3} mm^2/s for the slow component) are similar to those previously reported in brain slice preparations (0.96 ± 0.10 × 10^{-3} mm^2/s and 0.060 ± 0.006 × 10^{-3} mm^2/s, respectively) (7). However, if a two-compartment model is used, the relative distribution between the fast and slow compartments is different in heart slices compared with the brain slice preparation (0.82 ± 0.03 vs. 0.55 ± 0.05 for the fast component, respectively). As discussed in our previous work (6, 7), this may be due to differences in the T2 of the tissues. Cardiac tissue is known to have a T2 when fit to a monoexponential that is nearly half that of the brain (4), and one group (8) has measured the T2 in isolated rat hearts to be biexponential. Presuming the short T2 component to be intracellular, then a small ratio of the intracellular to the extracellular T2 would explain both the short average T2 obtained by a monoexponential fit and the increased volume fraction of the fast diffusing compartment. This speculation is further evidenced by the observation that Niendorf et al. (15) reported a fast diffusion compartment size of 0.80 ± 0.02 for healthy adult rat brain measured in vivo (i.e., larger than in the brain slice) but used longer echo times in their measurements which would also weight their results toward the longer T2 compartment. Further studies measuring T2 on isolated brain and heart slices are required, with cell volume perturbations and verification by alternate techniques. In addition, the heart has a larger vascular volume than that of the brain (14–22 vs. ~5%) (16, 22). Because the vascular compartment may contain perfusate with a longer T2 and diffusion coefficient it is likely to contribute to the fast diffusion component. Additional studies are underway to determine if the vascular compartment can be identified, e.g., if the diffusion data are triexponential (see Vascular component to diffusion).

Water exchange between the intra- and extracellular compartments may also influence our measurements. The signal obtained in a diffusion experiment is dependent on the T2 and diffusion coefficient of water in each compartment and the rate of water exchange between compartments (11). With the use of a simple tissue model (19) and previously estimated values for T2 of the intra- and extracellular spaces of the isolated perfused heart (8) we have simulated the influence of water exchange upon our measurements (Fig. 4). With a diffusion time of 11 ms and an echo time of 30 ms the difference between exchange rates of 0 and 27 Hz (estimated upper limit for cellular-interstitial exchange (8)) is negligible.

Nonmonoexponential diffusion has been observed in excised tissue in vitro using spectroscopic techniques (11) but no attempt was made to interpret these find-
ings. Although cardiac tissue was not examined, a biexponential fit to data from excised muscle (bovine quadriceps) gave fast and slow diffusion components with ADCs of $1.3 \times 10^{-3}$ mm$^2$/s and $0.07 \times 10^{-3}$ mm$^2$/s, and a fast component fraction of 96%. Bearing in mind that this bovine tissue was not perfused, not cardiac tissue, was examined at 25°C and with a different diffusion time of 20 ms, these data are comparable to those reported in this study on perfused heart slices. It is interesting to note that biexponential behavior was observed in several other tissue types in an in vitro study by Henkelman et al. (11). This implies that the compartmental nature of biological tissue, with respect to the water diffusion coefficient, may be generally applicable.

These observations also have important implications for both the interpretation of diffusion tensor measurements in myocardial tissue (12, 18), as well as the temporal changes that have been observed in the ADC of ischemic myocardial tissue (13). It is reasonable to assume that the small $b$-values that were applied in the previous determination of the eigenvalues and eigenvectors of diffusion in the heart (12, 18) reflect a general bias toward the fast diffusing component. However, information regarding the tensor orientations of the slow component remains unresolved. Furthermore, it is not known if the relative size of the diffusion compartments change under pathophysiological conditions. The progressive decrease in ADC observed in the isolated heart after ischemia could reflect a relative redistribution of water within the two diffusion compartments without a change in the diffusion coefficients of the compartments themselves. This would be similar to the changes recently reported in brain slices following administration of ouabain (6) and NMDA (7).

However, the apparent diffusion coefficient of the fast component in our isolated heart slice preparations is significantly less than the ADC values determined for the intact perfused arrested heart (10) even when temperature effects are taken into account ($1.1 \sim 1.8 \times 10^{-3}$ mm$^2$/s). This may reflect myofibrillar size and angle reorientation (resulting from either tissue sectioning or ischemia), increased cytosolic swelling of the slice preparation, or a collapse of the intravascular compartment in the slice preparation (reported to be $14 \sim 22\%$ of the myocardial volume) (16, 22).

**Myofibrillar orientation and cell size effects.** Although acknowledged to occur relatively rapidly (2 h) after myocardial ischemia (16), there is no evidence that a rearrangement of myofibrillar orientation occurs in the time frame encompassed by these studies ($\sim 35$ min). Moreover, the orientation of the diffusion gradients (along the z-axis) should result in relatively homogenous image intensity because the fiber orientation would be largely perpendicular to the gradients. This homogenous image intensity was observed in all of the tissue slice preparations that were examined, suggesting that gross rearrangements of myofibrillar orientation did not occur in this preparation (see Fig. 2). It is interesting to note that under pathophysiological conditions (e.g., hypertrophy, diabetes, or ischemia-reperfusion) changes in myocyte fiber orientation may complicate issues of analysis based solely on ADC calculations. Perhaps of greater concern to this study is the observation that the rabbit myocyte is $\sim 80\sim 100 \mu$m in length (16), which raises the possibility that a significant portion of the cells that border the cleavage planes for the ventricular slice preparation may be damaged. Because the tissue slice itself is $\sim 400\sim 800 \mu$m in thickness, a fraction of the cells will be compromised, and this will presumably be reflected by elevated values for the apparent diffusion coefficient of water in the tissue. However, by limiting the through-plane MR slice thickness to 300 m, we sought to minimize any contribution from damaged cells located along the cleavage planes. The observation that the fast component of the ADC that was measured is consistent with that previously reported at this temperature suggests we were successful in avoiding this potential artifact. Moreover, ADC measurements made in tissue slices isolated from papillary muscle (500–800 m) had ADC values that were not significantly different from the tissue slices isolated from the LV free wall (400–800 m).

**Cytosolic swelling in myocardial tissue.** Cell swelling within the myocardial tissue might be expected to lower the apparent diffusion coefficient, although work from this laboratory suggests that the ADCs of the individual compartments do not change with cytosolic swelling (6). Moreover, issues of cell swelling might be expected to become somewhat more relevant under normothermic conditions (i.e., 39°C), with tissues at the limit of diffusion for oxygen or metabolic substrate delivery (>500 m thickness), or under conditions of low or zero flow. This was not the case in these experiments. Combined with the decreased temperature, the presence of the cardioplegic solution results in very low oxygen consumption for this tissue (18). Recent diffusion estimates have also been reported using the excised canine right ventricle (12). The size of the right ventricular tissue sample that Hsu et al. (12) examined was $1.7 \times 2 \times 0.55$ cm, and the total time required for the study was 3.5 h. The tissue was rinsed and maintained in a chilled Ringer solution during the imaging protocol. In contrast, the LV free wall slices that were studied in this report were $0.4 \times 5 \times 5$ mm, and were superfused at a rate of 1 ml/min with well-oxygenated cardioplegic solution. Although the flow was turned off during the diffusion measurements, the entire diffusion imaging protocol did not exceed 35 min. Papillary slices tended to be somewhat thicker (500–800 m), yet did not result in diffusion coefficients that were different from those measured in the LV free wall, suggesting that viability was not compromised in the tissue samples that we studied.

**Vascular component to diffusion.** The report by Hsu et al. (12) examined the histological correlation of myofibrillar orientation with respect to the principle eigenvector of diffusion. This study was performed in excised (i.e., nonperfused) tissue samples of canine right ventricle at 20°C, and measured an average principle eigenvalue of $0.94 \pm 0.28 \times 10^{-3}$ mm$^2$/s. Subsequently,
our laboratory reported (18) histological validation of the correlation between the observed eigenvectors of diffusion with myocardial microarchitecture in the intact perfused rabbit heart. Although the measurements were made at the same temperature as those by Hsu et al. (12), the values determined for the principle eigenvalue ranged from 1.5 to $1.8 \times 10^{-3}$ mm$^2$/s (depending on their transmural position within the ventricular wall). Because the value reported by Hsu et al. (12) is similar to that reported in this study ($0.72 \pm 0.07 \times 10^{-3}$ mm$^2$/s), and both tissues were not perfused by direct vascular flow (although the tissue slice preparation was bathed in a exchanging nutrient- and oxygen-rich cardioplegic solution), it is possible that the decreased value for the diffusion coefficient that was observed in both studies might be due to the collapse of the vascular compartment before measurement. If this is true, then a more appropriate model for fitting diffusion in the intact myocardium might be a triexponential, rather than the biexponential model that was employed here. This will require a range of $b$-values employed to adequately sample the very fast, the fast, and the slow components of diffusion; these studies are currently underway.

In conclusion, multiexponential behavior has been observed for the first time in the diffusion coefficient of water in heart tissue. It is speculated that the diffusion is at least biexponential, and may reflect intra- and extracellular compartmentation. However, other compartments must be considered (e.g., vascular). Thus macroscopic MR measurements in tissues appear to provide quantitative compartmental information at the microscopic cellular level. These observations have major implications for the interpretation of diffusion tensor measurements in cardiac tissue and changes in the ADC following pathophysiological insults (e.g., ischemia).

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