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Prolongation of Proton Spin Lattice Relaxation Times in Regionally Ischemic Tissue from Dog Hearts

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Proton NMR techniques were used to characterize acutely ischemic myocardial tissue from the dog. Ligation of the left anterior descending coronary artery for 30 min resulted in a consistent prolongation of the proton spin-lattice relaxation times (T_1) in samples from regionally ischemic heart muscle when compared with T_1 values for nonischemic areas from the same hearts. The relative prolongation of relaxation times in ischemic tissue was found to increase as the duration of ischemia was extended to 60 or 120 min. T_1 values for ischemic tissue were not directly related to tissue levels of high-energy compounds, lactate, or hydrogen ions but largely reflected the increased water content of the regionally ischemic myocardium. Proton NMR analysis provides a means of identifying acute regional ischemia in heart tissue, and in the future may permit three-dimensional imaging of the heart in vivo.

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In 1974, Hoult et al. (*1*) demonstrated that nuclear magnetic resonance (NMR) techniques could be used to detect biochemical and physical changes in intact animal tissues. Recently these techniques have been applied to the study of the biochemical events associated with cardiac ischemia. Garlick et al. (*2*) used phosphorus-31 NMR spectra to identify the characteristic changes in the myocardial content of phosphorus-containing metabolites that occur during global ischemia in the rat, and Hollis et al. (*3*) used similar P-31 NMR measurements to identify the presence of regionally ischemic rabbit myocardium. It has been proposed that further refinement of these phosphorus-NMR techniques will allow location of the areas of cardiac ischemia (*3*).

We have chosen to assess the potential of proton-NMR analysis to identify regional myocardial ischemia, largely because of the potentially greater resolution of proton-NMR (tenfold) when compared with P-31 NMR measurements, and because of the potential to develop proton-NMR zeumatographic techniques (*4*) that would allow three-dimensional imaging of the intact regionally ischemic heart. Damadian (*5*) and others (*6,7*) have shown that proton spin-lattice relaxation times, which reflect the motional freedom of water molecules, are different in neoplastic and normal tissues; they propose that the differences result from changes in the content or structure of water within the neoplastic cells. Since ischemia also results in changes in tissue water content (*8,9*), we postulated that changes in proton-NMR measurements could be used to identify ischemic cardiac muscle. This communication describes the effects of regional myocardial ischemia in the dog on the proton longitudinal relaxation times of water.

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METHODS

Animal preparation and tissue sampling. Forty mongrel dogs with body weights of 17 ± 2 kg (mean ± s.e.m.) were used for these studies. The animals were initially anesthetized by i.v. injection of secobarbital sodium (35 mg/kg). Additional anesthetic was administered as needed. An endotracheal tube was inserted and respirations were maintained with a Harvard respirator (tidal

volume 0.4–0.5 liter, 18–22 cycles per min). The left external jugular vein was cannulated for infusion of 0.9% saline. The heart was exposed through a left thoracotomy and the pericardium was opened and retracted with sutures. In 28 dogs the left anterior descending (LAD) coronary artery was ligated immediately superior to its final major lateral branching. Terminal branches of the circumflex coronary artery, where they extended over the apex of the heart, were also ligated. After ligation, the sutures retracting the pericardium were cut, allowing the heart to rest in a more normal position within the chest. The heart was moistened with 0.9% saline solution and the wound was covered with moistened gauze.

The experiments were terminated and regional tissue biopsies were taken at 30 min following LAD ligation (nine animals), similarly at 40 min (four animals), 60 min (ten animals), or 120 min (five animals). Twelve control animals underwent similar surgery but with omission of the coronary ligations, the biopsies being taken immediately after exposure of the heart. The biopsy samples (6.3 ± 0.2 g) from control animals and from those with LAD ligation for 30 or 60 min were obtained rapidly from three areas of each heart: (1) the lateral base of the left ventricle; (2) the lateral right-ventricular wall, avoiding the area of the right ventricle supplied by branches of the LAD; and (3) the apical left-ventricular wall distal to the LAD ligation. In the animals subjected to LAD ligation, areas 1 and 2 were considered the nonischemic control regions, and area 3 the ischemic region. In the dogs subjected to 40 and 120 min of LAD ligation, biopsy of the second control area (area 2) was omitted. The tissue samples were blotted free of excess blood, placed in tared glass liquid-scintillation vials, and sealed with a double layer of parafilm. They were kept at room temperature for the NMR determinations. In selected animals, small specimens from areas 1 and 3 were freeze-clamped for metabolic studies. Also, in six experiments, biopsies were taken from the NMR tissue samples during the 60-min period used for the multiple T_1 measurements (see below).

NMR measurements. Proton nuclear magnetic longitudinal relaxation times were measured by the inversion-recovery technique (*10*). The nuclear magnetization induced by a static field of 2340 gauss was inverted by the application of a short pulse of radiofrequency magnetic field (180° pulse). After a variable time, τ, the magnitude of the magnetization was measured by application of a second radiofrequency pulse (90° pulse). The system was then allowed to return to its equilibrium magnetization and the sequence was repeated, using sequentially larger values of τ. Nuclear signals corresponding to 12–13 different values of τ were obtained by plotting the magnetization against τ. The relaxation behavior was obtained by fitting one or more exponentials (each defined by a relaxation time) to the data points collected during the relaxation recovery. The relaxation was dominated (over 95% of the signal amplitude) by a single relaxation time. For each sample, the measurement of relaxation time (T_1) was repeated four times over a 60-min period.

The 180° radiofrequency pulses (of 30 μ sec duration) were produced by a gated power amplifier* and gating modulator†. The nuclear signals were detected by a tuned receiver,‡ stored in a transient-recorder,‖ and plotted for analysis with an x-y recorder.

Percent water determinations. Following the NMR measurements, the parafilm was removed and the tissue samples were quickly weighed and placed back in the vials. The open vials were placed in a drying oven and dried to constant weight at 66–68°C. The samples were reweighed and the percent water in the original sample was calculated as (initial weight − dry weight) ÷ initial weight.

Metabolic determinations. Myocardial tissues from the control and the ischemic regions were rapidly frozen in liquid nitrogen. The samples were then powdered in a percussion mortar and deproteinized in ice-cold perchloric acid. The levels of lactate, adenosine triphosphate (ATP), and creatine phosphate were measured spectrophotometrically in the neutralized perchloric acid extracts by standard enzymatic procedures (*11–13*).

Statistical analysis. The data were analyzed by the paired-t and Student's t-test for the means of two populations where applicable. The data are presented as mean ± standard error of the mean.

RESULTS

Proton longitudinal relaxation times of myocardial tissue from 12 control dogs and from 28 dogs with LAD ligation are listed in Table 1. Three to 4 min were required to obtain the initial T_1 relaxation time for each sample of cardiac tissue. During the subsequent 60-min period, repeated T_1 measurements for each sample varied by less than 2%. The mean T_1 values of biopsies from areas 1, 2, and 3 from control dogs without coronary ligation were similar. Moreover, when T_1 values from the three areas of each heart were compared with one another, they varied by only 2.5% ± 0.4%.

Relaxation times for tissue from the two control areas (areas 1 and 2) of dogs with LAD ligation for 30–120 min were also similar to each other. However, the T_1 values of samples from the ischemic area (area 3) were consistently and significantly prolonged ($P < 0.01$) when compared by paired-t analysis with the values for nonischemic tissue from the same heart. To correct for the day-to-day differences in control values, which largely reflect biologic variability, the data are also expressed as the ratio between relaxation times for areas 1 and 3. In control dogs this ratio was 1.02 ± 0.01, and for dogs with 30 min of LAD ligation it was 0.95 ± 0.01 ($P < 0.01$). In dogs subjected to 60 min of ligation, the ratio

TABLE 1. EFFECT OF ACUTE REGIONAL ISCHEMIA ON MEAN LONGITUDINAL PROTON RELAXATION TIMES (T_1)

Duration of ischemia (min)	T_1 (msec)			
	Control regions		Ischemic region	
	Area 1	Area 2	Area 3	Area 1/Area 3
0	515 ± 5	499 ± 6	507 ± 6	1.02 ± 0.01
30	509 ± 6	512 ± 7	538 ± 8*	0.95 ± 0.01
40	495 ± 10	—	527 ± 10*	0.92 ± 0.02
60	491 ± 6	483 ± 13	541 ± 8*	0.91 ± 0.01
120	502 ± 8	—	551 ± 10†	0.91 ± 0.01

* Denotes $P < 0.01$ when compared with either area 1 or area 2.
† Denotes $P < 0.001$ when compared with either area 1 or area 2.

was further reduced to 0.91 ± 0.01 ($P < 0.01$ when compared with that in animals with 30 min of ligation). There was no significant difference between the values for animals with 60 and 120 min of ligation.

In dogs without coronary ligation, the percent water content was similar in each of the three biopsy areas (Table 2). Also, in the dogs with LAD ligation, the values for the two control areas of each heart were not significantly different from each other. As predicted, the percent water content of the ischemic-area tissue was significantly increased ($P < 0.01$) compared with that of nonischemic tissue from the same heart (Table 2). The changes in the ratio of percent water content for areas 1 and 3 with increasing duration of ischemia agreed with those observed for the ratio of T_1 values for the same areas.

To study the relationship between the T_1 values and metabolic indices of cell function, we determined the levels of high-energy compounds and lactate in myocardial tissues from areas 1 and 3 of the dogs subjected to LAD ligation for 40 or 120 min. As expected, ATP and creatine phosphate were significantly diminished, and there was a fourfold increase in the lactate content of area-3 tissue compared with control areas from the same hearts (Table 3). However, the data in Table 3 were obtained from tissue rapidly frozen at the time the samples were taken for T_1 measurements. In addition, in six experiments we performed similar biochemical studies using small biopsies from the NMR tissue samples (areas 1 and 3) that were undergoing repeated T_1 measurements at room temperature over a 60-min period. The myocardial contents of ATP, creatine phosphate, and lactate continued to change during this period. Figure 1 depicts a representative experiment from a heart that had been subjected to 40 min of regional myocardial ischemia. ATP and creatine phosphate levels of both control and ischemic areas declined, and there was a two- to eightfold increase in tissue lactate during the NMR analysis. During this 60-min period the T_1 values were constant.

DISCUSSION

The present study was designed to evaluate the effects of regional myocardial ischemia on proton NMR parameters. The data presented show that proton longitudinal relaxation times (T_1) of water obtained from acutely ischemic tissue were increased when compared

TABLE 2. EFFECT OF ACUTE ISCHEMIA ON MEAN PERCENT WATER CONTENT* OF REGIONAL MYOCARDIAL TISSUE

Duration of ischemia (min)	Control regions		Ischemic region	
	Area 1	Area 2	Area 3	Area 1/Area 3
0	76.9 ± 0.3	76.9 ± 0.3	76.9 ± 0.4	1.00 ± 0.01
30	76.6 ± 0.3	76.3 ± 0.4	77.7 ± 0.3†	0.98 ± 0.01
40	76.3 ± 0.8	—	77.5 ± 0.8†	0.98 ± 0.01
60	76.3 ± 0.3	76.4 ± 0.5	78.8 ± 0.4‡	0.97 ± 0.01
120	77.0 ± 0.2	—	79.4 ± 0.4‡	0.97 ± 0.01

* Percent water content is expressed as (initial weight − dry weight) ÷ initial weight.
† Denotes $P < 0.05$ when compared with values for area 1 or area 2.
‡ Denotes $P < 0.01$ when compared with values for area 1 or area 2.

TABLE 3. EFFECT OF ACUTE REGIONAL ISCHEMIA IN THE DOG ON MYOCARDIAL CONTENTS OF LACTATE, ADENOSINE TRIPHOSPHATE, AND CREATINE PHOSPHATE

Duration of ischemia (min)		ATP	CP	Lactate
		(μmol/g wet wt)		
40	Area 1	4.5	4.5	1.0
(n = 4)		(3.1–5.3)	(2.2–5.9)	(0.4–2.0)
	Area 3	1.9*	0.5*	6.5*
		(1.2–3.9)	(0.4–0.5)	(1.6–10.2)
120	Area 1	4.7	5.2	1.7
(n = 4)		(4.2–5.4)	(3.7–7.5)	(0.9–3.4)
	Area 3	0.7*	1.6*	9.1*
		(0.5–0.9)	(1.2–2.2)	(5.3–12.0)

* Denotes $P < 0.02$ when compared with values for area 1.

with values obtained from nonischemic tissues from the same hearts. We stress that although the increase in relaxation time in the ischemic tissue was small, it was consistently present and highly significant when compared with control values from the same heart. A similar increase in T_1 values in heart tissue made ischemic for 4 hr was reported by Frank et al. (*14*).

The mechanism underlying the increase in T_1 values in acutely ischemic heart tissue is not precisely known. Proton longitudinal relaxation times are a function of the net magnetic field that the proton nucleus experiences. This field is generated from both the applied magnetic fields and the intrinsic magnetic forces arising from the local molecular environment. The local magnetic field of ischemic heart muscle may be altered as a result of one or more of the complex physical and biochemical changes that occur during myocardial ischemia. These include (a) changes in the tissue content of oxygen, high-energy compounds, and cations; (b) changes in the content and distribution of tissue water; and (c) changes in pH (*15*).

The prolongation of T_1 values in ischemic heart tissue was not directly related to changes in the tissue content of oxygen, carbon dioxide, and hydrogen ions, since the T_1 values from the control tissues remained constant during repeated measurements over a 60-min period. During this period the control tissues were most certainly hypoxic. Similarly, the fact that the myocardial levels of ATP, CP, and lactate continued to change during this 60-min period showed that the ischemia-associated alterations in T_1 values were not directly related to these metabolic parameters.

Jennings et al. (*8*) have demonstrated that myocardial ischemia following acute coronary artery ligation results in a time-dependent increase in tissue content of water and sodium, and we observed a similar increase in the water content of ischemic tissue in this study. The increase in the T_1 of ischemic tissue, reflecting the motional freedom of water protons, is likely due in large part to this increase in tissue water content. It is of interest that although there was a positive correlation between the mean water content and the spin-lattice relaxation times, the T_1 changes exceeded the water content changes as the duration of ischemia was increased. It has

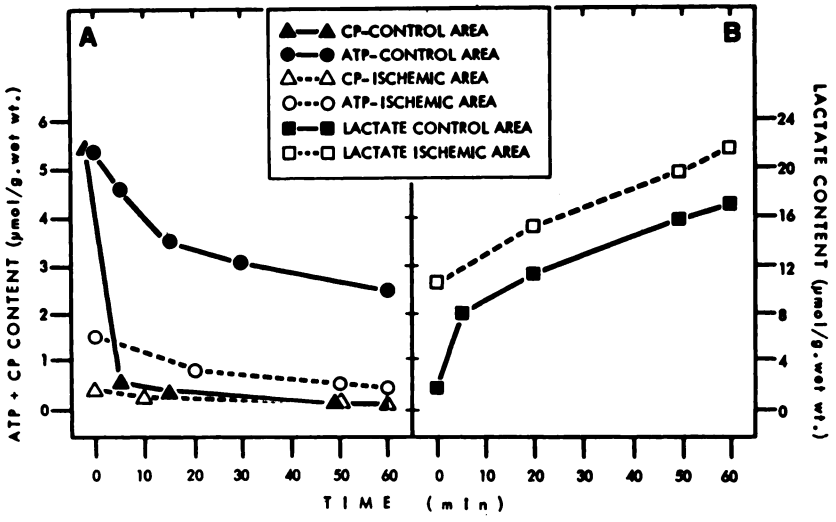

FIG. 1. Myocardial levels of ATP and creatine phosphate (A) and lactate (B) in tissue samples used for proton-NMR measurements. Time scale shows time after excision of samples from heart.

been proposed (*16*) that the decay in proton longitudinal relaxation that follows global ischemia in the liver can best be described by a two-compartment model in which there is relatively slow exchange of water molecules between intracellular and extracellular compartments. This model predicts that the average relaxation rate depends upon both the total water content and the relative amounts of water in the slow-relaxing and the fast-relaxing compartments.

Raaphorst et al. have demonstrated that changes in the ratio of sodium to potassium may alter proton NMR relaxation times in mammalian cells (*17*). This presumably results from differences in the hydration shells of the sodium and potassium ions and differences in the effects of the two ions on the coordination shells of macromolecules. Thus, as the relative contents of the two ions are changed, the motional freedom of water protons is also changed. Regional myocardial ischemia results in a flow of potassium out of the intracellular compartment, and Hill and Gettes (*18*) have recently demonstrated with an ion-sensitive electrode that the interstitial concentration of potassium may reach 10- to 13 millimolar levels within minutes following ligation of the LAD coronary artery. Thus, changes in the sodium and potassium contents and distribution may contribute to the prolongation of ischemic-tissue T_1 values in our studies.

The observation that T_1 relaxation times are different in ischemic and nonischemic tissues from the same heart is particularly intriguing in light of the recent development of proton NMR zeumatographic techniques, which have been used to obtain three-dimensional sample imaging (*4*). If these techniques can be applied to the heart, it will become possible to localize rapidly and to quantify ischemic regions of the heart in vivo. Such mapping would not require injection of radioactive material and would allow sequential studies of regional ischemia. Although other nuclear species (e.g., P-31) could theoretically be used for three-dimensional imaging, the relative abundance of protons in myocardial tissue will permit proton-NMR studies to utilize low magnetic fields and will yield greater mapping resolution. The finding that proton spin-lattice relaxation times are consistently altered in regionally ischemic hearts is an important first step toward application of this technique. Studies to characterize the sensitivity of the ischemia-associated changes in T_1 values are currently in progress in our laboratory.

FOOTNOTES

* Matec Model 515,
† Matec Model 5100
‡ Matec Model 615
‖ Biomation

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