In Vitro Magnetic Relaxation Times of the Ischemic and Reperfused Rabbit Kidney: Concise Communication

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To assess the effects of renal ischemia and reperfusion on in vitro magnetic relaxation times (T_1 = magnetization recovery, T_2 = spin echo), we evaluated the spectroscopic characteristics of the renal cortex from 25 rabbits. Eight served as controls (Group 1), nine had one renal pedicle ligated for 1 hr (Group 2), and eight (Group 3) were occluded for 1 hr and reperfused for 30 min. For intraanimal comparison purposes, % H_2O content, T_1 (msec), and T_2 (msec) of the ischemic (reperfused) kidney were normalized to the values from the normal kidney within the same animal. Renal ischemia consistently increased water content, which was exaggerated by reperfusion. In association with ischemia, T_1 fell, and with reperfusion T_1 lengthened. T_2 increased with ischemia and declined from the peak ischemic effects with reperfusion.

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Regulation of cell volume depends at least partially on the supply of metabolic energy that prevents particular ions (especially sodium) from diffusing down metabolically maintained concentration gradients (*1–3*). Both hypovolemia (hemorrhage, burns, etc.) and cardiovascular failure (infarction, tamponade, vascular pooling during shock) characteristically result in acute and chronic renal dysfunction (*4*). In ischemic renal injury, isolated tubular cell necrosis is rare. There tends to be associated disruption of the basement membrane and brush border, along with mitochondrial dissolution and the development of intracytoplasmic inclusion bodies (*5*). The interstitium becomes edematous, with cellular interstitial infiltrates (*4*). A typical clinical situation where renal ischemia and reperfusion result is in the postoperative or transiently hypotensive patient with briefly reduced renal perfusion and prolonged renal dysfunction.

Nuclear magnetic resonance (NMR) spectroscopy and imaging have the capacity to assess sequential changes in proton density and magnetic relaxation times. The utility and sensitivity of these techniques in detecting acute renal ischemic insults remain undetermined. Thus, the purposes of this study were: (a) to evaluate the effects of 1 hr of renal ischemia on magnetic relaxation times; (b) to examine the effects of renal reperfusion on renal water content and relaxation times, and (c) to compare the injured kidney with the corresponding normal kidney in the same animal.

METHODS

Experimental preparation and protocol. All experiments were performed on rabbits weighing between 2.5–3 kg. Twenty-five rabbits were randomly divided into three groups.

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Group 1 (N = 8) served as the control group and was killed with an i.v. injection of pentobarbital. The postmortem kidneys were then removed through a midline laparatomy for tissue analysis. Each kidney was dissected free from surrounding capsular tissue before tissue preparation.

Group 2 (N = 9) was anesthetized with 200 mg of subcutaneous ketamine and 15 mg of morphine sulfate. They were ventilated through an endotracheal tube placed through a midline tracheostomy. Supplemental anesthesia was administered as required. Through a midline laparotomy, both renal pedicles were isolated, and one was clamped for 1 hr. This included occlusion of the renal artery, vein, and ureter. All collateral (capsular and other) vessels were ligated. One hour after ligation, the animals were killed with an overdose of pentobarbital.

The third group of animals (Group 3, N = 8), was prepared identically to Group 2, only special vascular clamps were used to clamp one renal pedicle. One hour later, the clamp was removed, and blood flow restored (documented at necropsy by slicing the artery and demonstrating vascular patency) for 30 min. As above, the animals were killed with an i.v. overdose of pentobarbital. All studies were performed with the animals supine.

Nuclear magnetic resonance (NMR) spectroscopic analysis. Upon sacrifice, the cortex of each kidney (50 total kidneys) was diced into small (<0.1 cc) pieces, which were packed into a 10 (o.d.) × 75-mm Pyrex tube. Approximately (± 5%) 1 cc total volume of renal tissue was placed into each tube, and tissue analysis was performed (at room temperature) within 1 hr of death.

All data were obtained with a pulsed NMR device that uses a permanent 0.25 tesla (10.7 MHz) magnet and has a 10-mm probe sample size. A radio-frequency coil is located at the midpoint of the probe (4 μsec pulse width with 12 μsec dead time). Data acquisition was controlled by a microcomputer. Data and relaxation curves were displayed on a video unit, and on a dot matrix printer.

A number of preliminary studies were performed to determine the optimal tube location in the RF coil and magnetic field to maximize signal intensity (free induction decay peak). The selected volume of tissue examined (1 cc) was used because small alterations of tissue volume did not alter the FID peaks significantly. (The sample extended slightly above the RF coil.)

The following reproducibility studies were performed. To assess the affects of geometry on relaxation times, T_1 (spin-lattice) and T_2 (spin-spin) were calculated on 15 tissue samples five times in succession. Data were then reacquired after sequentially turning the test tube 90°, 180°, 270°, and 360°. The coefficient of variation (100 × standard deviation/mean) of these experiments was <1% for T_2 and <2% for T_1 (in an individual tissue).

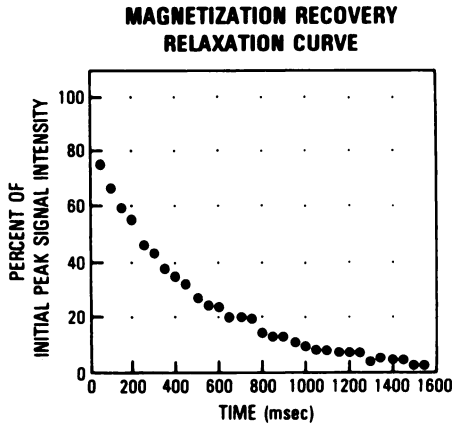

FIG. 1. Typical T_1 (magnetization recovery) curve. Each point represents average of two data points.

Each T_2 measurement required 3 min, and each T_1 measurement 10 min.

T_1 was obtained using a standard magnetization recovery approach (180 t 90) (*6*). The positive peaks of the induction decay curve were plotted. Each curve was composed of >60 points, each displayed point being the average of two data points. Each point was derived as an average of three separate pulsing sequences, with an interpulse delay of 5 sec to ensure complete magnetization recovery before repulsing. Each data curve actually represented a plot of

$$\frac{S_o - S_t}{S_o} = ae^{-T/T_1}$$

for many different values of T (tau), and peak intensity (S). A least-squares monoexponential curve was fitted to the downslope of the free induction decay curve, and T_1 derived as the inverse of the slope rate. The correlation coefficient of each exponential curve fit exceeded 0.98. T_2 was derived from the spin-echo technique (90 τ 180) (*7*), using five echoes per point and 500-msec pulsing delays. Similar curve analysis (peaks of the free induction delay) fitting was used for T_2 calculations (see Figs. 1 and 2).

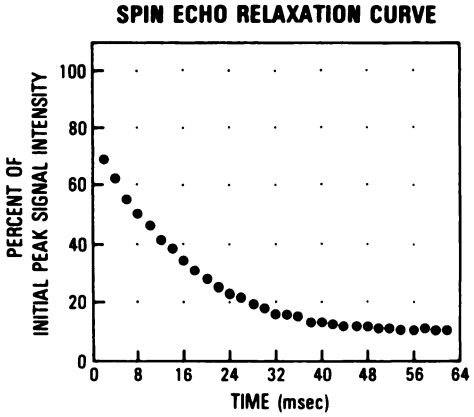

FIG. 2. Typical T_2 (spin-echo) curve. Each dot represents average of two data points.

TABLE 1. RENAL ISCHEMIA AND REPERFUSION

	% H_2O content	T_1 (msec)	T_2 (msec)
Control (n = 33)	0.74 ± 0.03	489 ± 69	51.7 ± 1.6
Ischemia (n = 8)	0.77 ± 0.02	456 ± 38	57.9 ± 2.0
Reperfusion (n = 8)	0.80 ± 0.03	567 ± 31	55.1 ± 3.1

Analysis of tissue water content. Wet weights were obtained for each sample before NMR analysis. After the analysis, each sample was gently heated in a vacuum oven. After 7 days of drying, each tube was reweighed. Percent water content was defined as:

$$\frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100$$

Statistics. All data are given as the group mean ± one standard deviation unless otherwise specified. Comparisons were made with an analysis of variance.

RESULTS

Water content (Table 1 and Fig. 3). The normal kidneys were (74 ± 3)% water by weight, significantly less than the ischemic [(77 ± 2)%, $p < 0.05$] and reperfused kidneys [(80 ± 3)%, $p < 0.01$ compared with control].

T_1 relaxation (magnetization recovery) times. The normal T_1 (msec) was 489 ± 69 msec. With occlusive ischemia, T_1 actually declined slightly (456 ± 38 msec), increasing with reperfusion (567 ± 31 msec, $p < 0.01$). See Table 1 and Fig. 4 for a graphic display of the data.

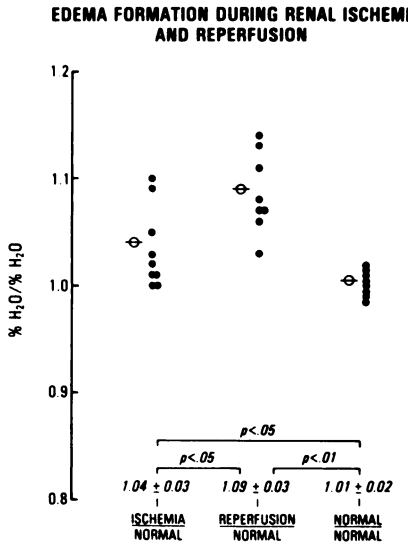

FIG. 3. Water changes in each kidney are shown, normalized to control kidney in each animal.

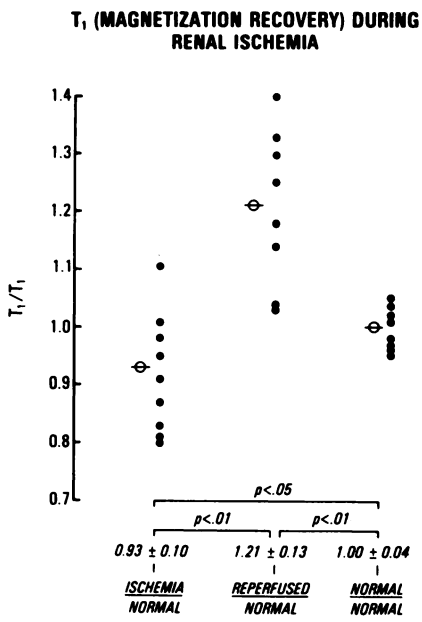

FIG. 4. As in Fig. 3, normalized T_1 data are shown for each of three groups.

T_2 relaxation times (spin-echo). The normal T_2 was 51.7 ± 1.6 msec, which increased with ischemia (57.9 ± 2.0 msec, $p < 0.01$), then declined with reperfusion (55.1 ± 3.1 msec). The comparative data are plotted in Fig. 5.

In the present study, the correlation between T_1 and % H_2O was 0.68 ($p < 0.01$), and between T_2 and % H_2O, 0.71 ($p < 0.01$).

CONCLUSIONS

Renal ischemia results in a gradual increase in renal water content, which is exacerbated by renal vascular reperfusion. In conjunction with these pathophysiological interventions, T_1 first decreases (ischemia) and then

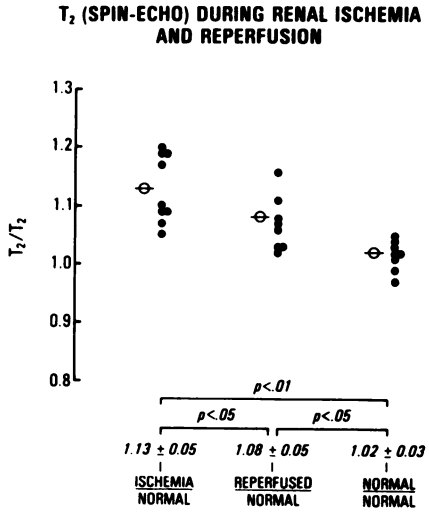

FIG. 5. Normalized T_2 data are shown for each of three groups.

increases (reperfusion). The specific causes of these changes remain unclear. With reperfusion, both water content and T_1 relaxation time increase significantly. Importantly, we evaluated the occlusion of the renal artery, vein, and ureter simultaneously. Arterial, venous, and ureteral occlusion will need to be evaluated separately before we can sort out the individual effects (*8*).

After muscle infarction (with intravenous ethanol), Herfkens et al. (*9*) demonstrated increases in both T_1 and T_2 relaxation times. We have made similar observations during myocardial ischemia. In both of these experimental situations, residual or collateral flow is available to the organ in jeopardy, in contrast to the present model of isolated renal ischemia. Nonetheless, in each of these three studies (myocardial, muscle, and renal ischemia), the elevation in T_2 relaxation time was an acute marker of damage. We have also observed similar qualitative changes in acutely ischemic lungs.

While the present study was performed as an in vitro tissue study, Hriack and co-workers (*10,11*) have repeatedly demonstrated that NMR renal imaging is simple to perform and relaxation parameters easy to derive from modern imagers.

A variety of limitations of in vitro spectroscopy should be pointed out. The tissue sample is at room temperature, and there is no "flowing blood" in the "field of view." It is impossible to control the changes in the bound-to-unbound water ratios that result over time. However, in airtight samples, we have seen little change in T_1 or T_2 for as long as 5–6 hr after initial data acquisition and analysis. Similar observations have been made by Thickman et al. while evaluating in vitro relaxation characteristics of excised tissue in a proton spectrometer (*12*).

In the present study, reperfusion of an ischemically damaged kidney resulted in a dramatic increase in T_1 when compared with a nonreperfused ischemic kidney. This observation has direct, and important, implications for the imaging of the anuric patient after operation, hemorrhage, trauma, or atherosclerotic occlusion.

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