Interstitial sodium nuclear magnetic resonance relaxation times in perfused hearts

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ABSTRACT Separation of intracellular and extracellular sodium nuclear magnetic resonance (NMR) signals would enable nondestructive monitoring of intracellular sodium. It has been proposed that differences between the relaxation times of intracellular and extracellular sodium be used either directly or indirectly to separate the signal from each compartment. However, whereas intracellular sodium relaxation times have been characterized for some systems, these times were unknown for interstitial sodium. In this study, the interstitial sodium NMR relaxation times have been measured in perfused frog and rat hearts under control conditions. This was achieved by eliminating the NMR signal from the extracardiac (perfusate) sodium, and then quantifying the remaining cardiac signal. The intracellular signal was measured to be 8% (frog) or 22% (rat) of the cardiac signal and its subtraction was found to have a negligible effect on the cardiac relaxation times. Therefore this cardiac signal is considered to provide a good estimate of interstitial relaxation behavior. For perfused frog (rat) hearts under control conditions, this signal was found to have a T_1 of 31.6 ± 3.0 ms (27.3 ± 1.6 ms) and a biexponential T_2 of 1.9 ± 1.0 ms (2.1 ± 0.3 ms) and 25.2 ± 1.3 ms (26.3 ± 3.2 ms). Due to the methods used to separate cardiac signal from perfusate signal, it is possible that this characterized only a part of the signal from the interstitium. The short T_2 component attributable to the interstitial signal indicates that separation of the NMR signals from each compartment on the basis of relaxation times alone may be difficult.

INTRODUCTION

The sodium ion is involved in numerous physiological and pathological processes. Cell death, edema formation, tumor growth, electrophysiologic processes, and ion transport all involve alterations in intracellular and extracellular sodium levels. By observing intracellular sodium, the state of the Na-K ATPase pump and other ion transport mechanisms can be monitored. By observing extracellular sodium, one is able to monitor extracellular volume, because the sodium concentration in the extracellular space remains constant. In this way, the presence of extracellular edema can be detected. Nuclear magnetic resonance (NMR) provides a nondestructive method of observing sodium; however, it is difficult to separate the intracellular sodium signal from the extracellular sodium signal. Several NMR methods have been proposed to obtain this compartmental sodium information, but each has certain difficulties. The proposed methods include the use of shift reagents, differences between the sodium NMR relaxation times in these compartments, and multiple quantum spectroscopy.

Shift reagents are chemicals which are added to a system of biological interest and which penetrate only the extracellular spaces (1, 2). The shift reagent alters the NMR resonant frequency of the extracellular cations which means that the intracellular and extracellular signals occur at different resonant frequencies, enabling the signal from the two compartments to be separated. However, problems exist with the in vivo use of shift reagents. Shift reagents chelate calcium and thus may be toxic to functioning organs (2–4), and shift reagents may not be distributed uniformly throughout the body, such as through the blood-brain barrier, in necrotic centers of tumors, or in underperfused tissue.

Differences in the NMR relaxation times between intra- and extracellular sodium have also been proposed as a means of obtaining compartmental sodium information (5). The intracellular relaxation times have been measured with shift reagent for frog hearts and were found to be 23 ms for T_1, and 2 and 16 ms for a biexponential T_2 (6). The relaxation times of extracellular fluids have also been measured in vitro (7) and were found to be longer than the intracellular times (54 ms for T_1 and 53 ms for T_2 of cerebrospinal fluid; 37 ms for T_1 and 24 ms for T_2 of plasma). With this information in mind, techniques have been devised which detect or emphasize only the signal with short relaxation times, such as the 2 ms T_2 component of intracellular sodium. If the intracellular compartment is the only compartment with such short relaxation times, then these techniques would enable the selective monitoring of intracellular

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sodium. However, the relaxation characteristics of sodium present between the cells, the interstitial sodium, are unknown. Thus one is not sure whether techniques which emphasize short relaxation times are selectively detecting intracellular sodium, or whether contributions from both intracellular and interstitial sodium are present.

The final method recently proposed to address the problem of separating the sodium signal from the intracellular and extracellular compartments is to utilize multiple quantum spectroscopy (MQS) (8). This technique can selectively yield signal from compartments which exhibit biexponential T_2 decay as a result of quadrupolar relaxation. However, because the characteristics of interstitial sodium have not been determined, it is unknown whether interstitial sodium contributes to the multiple quantum signal. Very recently, the combination of MQS and paramagnetic relaxation reagents to "quench" any contribution of extracellular sodium has been proposed (9). This approach is still under investigation for its use in perfused organs or in vivo applications. It does still require the use of an exogenous agent whose distribution in the body may not be uniform.

Clearly a major difficulty in relaxation-based methods and MQS is the lack of NMR information concerning interstitial sodium. In this work, techniques have been devised to determine the relaxation characteristics of interstitial sodium in perfused hearts. These techniques are tested on phantoms and then applied to perfused frog and rat hearts under control conditions.

THEORY

Sodium-23 has a nuclear spin of 3/2. Therefore, in the presence of a magnetic field, the sodium nucleus can be in one of four possible energy states, with three equally spaced transitions between them. Sodium-23 also possesses a quadrupolar moment, as do all nuclei with nuclear spin greater than one-half. In the presence of an electric field gradient, the interaction of the electric field gradient with the quadrupolar moment causes a perturbation of the energy levels such that the transitions are not equally spaced. This would produce frequency splitting of the sodium NMR spectrum. However, the NMR techniques used may not be sensitive enough to detect the signal at all of the frequencies. In addition, if fast isotropic motions of the sodium nuclei average out the electric field gradient at the nucleus over the course of a measurement, the signal would occur only at one central resonant frequency. In physiological systems, only one resonant frequency has been detected. It is the signal at this single detectable frequency which is studied here.

The presence of multiple transitions between energy levels leads to the possibility of multiexponential relaxation behavior, even if all of the sodium nuclei are in the same environment or "pool." For a homogeneous pool of sodium ions with a single resonant frequency, theory predicts that both T_1 and T_2 relaxation behavior will be biexponential with a ratio of fast to slow components of 0.2 to 0.8 for T_1 and 0.6 to 0.4 for T_2. The two relaxation time constants for T_1 are difficult to separate experimentally, due both to the small fraction of the fast component and to the small difference predicted for the two T_1 values. Thus a single T_1 time constant is usually determined from the data. For T_2 data, multiexponential relaxation behavior is more obvious, and two relaxation time constants are frequently necessary to characterize the relaxation decay.

Quadrupolar relaxation, due to an interaction of the quadrupolar moment of the sodium nucleus with electric field gradients, has been shown to be the predominant relaxation mechanism for sodium (10). Quadrupolar relaxation is quite effective, leading to short T_1 and T_2 values. If the motion of the sodium ion is restricted in some fashion, such as by a gel matrix or large negatively charged molecules, then the possibility exists that the fast (60%) component of T_2 may be NMR "invisible" in that it relaxes too quickly for the receiver to detect it.

The above analysis is for a homogeneous pool of sodium ions. If two or more pools of ions are present, then the rate of exchange of ions between the pools becomes a factor in determining the overall relaxation behavior. A fast exchange will produce a weighted average of the relaxation times, whereas a slow exchange will produce a superposition of each compartment's relaxation behavior. With this additional complexity, many T_1 and T_2 time constants can be present in a physiological system, which has multiple compartments and environments with unknown exchange rates between these pools of sodium ions. Therefore the percent of the total signal which may be "invisible" is unpredictable.

In this study, the relaxation time data were fit to equations of a single exponential time constant for T_1 and two exponential time constants T_2. Because of the above considerations, this must be considered to be a characterization of the T_1 and T_2 decay of the system. The underlying physical distribution of relaxation times may be much more complex.

MATERIALS AND METHODS

Phantoms

One phantom consisted of a 20-mm o.d. NMR tube of 150 mM NaCl. A second phantom consisted of a latex balloon placed on the end of the perfusion cannula. A small hole was cut in the top of the balloon to allow the fluid which enters at the cannula to escape. This phantom was used to characterize the T_1 and T_2 behavior of flowing fluid in the perfusion apparatus. A third phantom consisted of an agarose/NaCl gel placed

inside a 15-mm o.d. NMR tube, which was placed inside a 20-mm o.d. NMR tube filled with 150 mM NaCl. The gel was prepared by placing 4 g agarose into 100 ml of 150 mM NaCl. The solution was stirred and heated until the agarose dissolved and then poured into a 15-mm o.d. tube and allowed to cool until the gel formed. The gel's volume approximated the volume of a perfused heart, which was ~1–2 ml for both frog and rat hearts. The entire 15-mm o.d. tube was then submerged into a larger 20-mm o.d. tube which had been partially filled with 150 mM NaCl. Both tubes were then sealed. This phantom provides a reasonable "relaxation-time model" of the perfused heart system with agarose representing the heart tissue as a substance having shortened relaxation times, and the NaCl solution representing the perfusate as a substance having long relaxation times. The advantage of the phantom is that the sodium relaxation times of the agarose can be measured separately, thus providing a way of validating the capability of the NMR techniques to quantify the relaxation times of one component (agarose) when the relaxation times of the other component (NaCl solution) are known, but the relative magnitudes of the two components are not known.

Physiological preparation

The studies were performed with perfused heart preparations as previously described for frogs (11) and rats (12). Briefly, bullfrogs (*Rana catesbeiana*) or rats (Sprague-Dawley) were anesthetized with pentobarbital by intraperitoneal injection. The frog (rat) heart was excised, cannulated at the atria (aorta), and perfused with Ringers (Krebs Henseleit) buffer at room temperature. The cannulated heart was mounted inside a 20-mm o.d. NMR tube, and the cardiac efflux was suctioned from below the level of the heart.

For frogs, the flow of perfusate from atria to ventricle mimics the normal blood flow through the heart as frog hearts do not possess coronary arteries. The flow rate for frog hearts was set to a constant value of 8 ml/min and heart rate and pressure were monitored by cannulating the aorta with a water-filled line connected to a pressure transducer. For rats, a Langendorff, nonworking preparation was used. A perfusion pressure of 100 cm of H_2O determined the flow rate for rats, which varied between 7 and 18 ml/min for different hearts. A balloon connected to a pressure transducer by a water-filled line was inserted into the left ventricle to enable monitoring of ventricular contractions.

After perfusion with normal Ringers or Krebs Henseleit buffer, during which time the interstitial relaxation measurements described below were obtained, several frog and rat hearts were also perfused with a buffer containing dysprosium tripolyphosphate [$Dy(PPP)_2^{-7}$] shift reagent (1) to quantify the intracellular signal. The composition of this buffer for the frog hearts is described elsewhere (11), whereas for the rat hearts the buffer was identical to the Krebs Henseleit solution except for the presence of 3 mM $Dy(PPP)_2^{-7}$ and an increased $CaCl_2$ concentration of 3 mM. After ~0.5 h of perfusion with this shift reagent, the signal stabilized and NMR measurements of the intracellular magnitude were obtained. The hearts which were perfused with shift reagent were returned to normal perfusion before being removed from the perfusion apparatus.

After completion of perfusion experiments, the hearts were removed from the cannula apparatus, sliced into two halves to expose the ventricle, blotted free of excess fluid for 30 s on paper towel, and then placed into a clean 20-mm o.d. NMR tube and reinserted into the magnet for further measurements as described below. The time elapsed from removal of the heart from the cannula to the beginning of further NMR measurements was never >3 min. After these measurements, the hearts were weighed.

Interstitial relaxation time measurements

The experiments were all performed at room temperature on a model 360 AM spectrometer (Bruker Instruments, Inc., Billerica, MA) operating at 95.262 MHz for sodium-23, with a broad-banded 20-mm probe. Typical 90° pulse widths were 30 μs, and the smallest echo time used was 0.32 ms.

To determine the relaxation characteristics of interstitial sodium, signal from intracellular sodium and extracardiac (perfusate) sodium must be taken into account. The method described below is utilized to eliminate the perfusate signal; the remaining (cardiac) signal consists of the intracellular plus interstitial signals. The effect of the intracellular signal on the relaxation characteristics of the total cardiac signal is described below.

To measure cardiac relaxation times, the following pulse sequence is used:

$$180^* - ID - 90° - TE/2 - 180° - TE/2 - AQ - RD,$$

where 180* represents a composite inverting pulse (13), *ID* is an inversion delay, *TE* is the echo time, *AQ* is acquisition of the signal, and *RD* is the recycle delay. A composite inverting pulse is used to reduce the effects of radio frequency inhomogeneity. This sequence is an inversion pulse followed by a delay and then a standard Hahn spin echo pulse sequence. It has been applied to other multicompartment systems for the purpose of separation of the signals arising from each compartment on the basis of relaxation times (14, 15). It will be called the Inversion Hahn Echo (IHE) sequence.

By setting *ID* (or *TE*) at a given value and varying *TE* (or *ID*), the IHE sequence is run under three conditions (described as separate experiments) to obtain data which emphasizes cardiac T_1 and T_2 decay. The data from these three experiments constitute a single data set whose analysis is described later. The experiments are: (*1*) T_2 measurement emphasizing cardiac signal; $ID = t'$, vary *TE*; (*2*) T_1 measurement of total perfused heart system; vary *ID*, $TE = 0.32$ ms; (*3*) T_2 measurement of total perfused heart system; no inverting pulse (equivalent to an *ID* of infinity), vary *TE*.

Experiments 1–3 are obtained in a cycled fashion such that a fraction of the averages required for experiment 1 are obtained, then experiments 2 and 3 are partially completed, then the second cycle begins with another fraction of the averages for experiment 1. Typically, eight cycles are used. The purpose of this is to average small changes in temperature or the heart's condition which may occur during the course of the experiment. This is necessary to enable combination of the experiments into a single data set.

Experiment 1 provides information about the cardiac T_2 decay. If one assumes that the cardiac signal has a component with a T_1 less than that of the perfusate, then, as described in Fig. 1, it is possible to choose ID such that perfusate signal is greatly reduced in magnitude. If a Hahn echo experiment begins at this ID time, then the T_2 data obtained is predominantly due to any components having a short T_1. By fixing ID to this value and varying TE, information on the T_2 decay of the cardiac signal can be obtained if it possesses a T_1 shorter than that of the extracardiac perfusate. Because the signal cancellation is not perfect (due to imperfect knowledge of t'), the T_2 time constants reported here were determined from the total fit of all three experiments as described below and not from experiment 1 alone; however, similar results for cardiac T_2 were obtained if the data from only experiment 1 were analyzed.

Determination of the optimal value for t' is done by two methods: (*a*) The T_1 behavior of the perfusate is determined in a separate experiment involving perfusate alone. (*b*) Using the IHE pulse sequence, the signal at *ID* values near t' and a long *TE* value is measured for each perfused

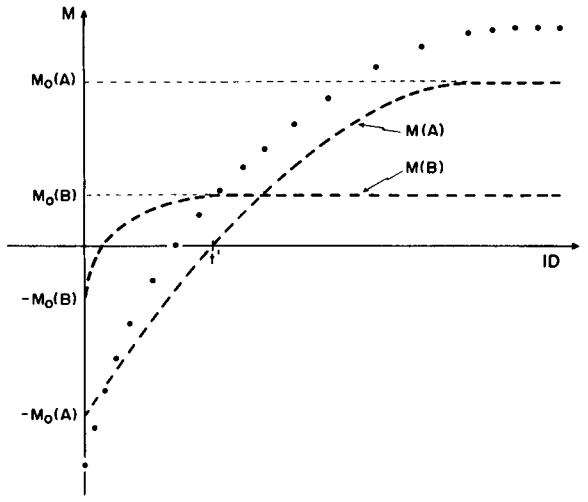

FIGURE 1 Diagram illustrating the application of the Inversion Hahn Echo pulse sequence to the elimination of perfusate signal. Component labeled $M(A)$ represents the T_1 recovery curve of the perfusate, with a large amount of signal and a long T_1 time constant. Component $M(B)$ has less signal and a shorter T_1 value and represents the possible behavior of a component of the cardiac signal. Dots are the sum of these two curves. Observation of the signal at the time labeled t' will enable selective detection of the signal from compartment B, because the signal from compartment A is nulled at this point. A Hahn echo sequence which begins at time t' will therefore enable the measurement of T_2 for component B.

heart preparation. As the perfusate possesses the longest T_2 value, the signal at a long TE value is assumed to be dominated by the perfusate. The ID value which eliminates this signal will thus null the perfusate signal.

Experiment 2 measures the T_1 recovery curve for the entire perfused heart system. It utilizes the IHE pulse sequence with multiple ID values and a fixed echo time of 0.32 ms. The T_1 recovery curve of experiment 2 provides information about the T_1 of the cardiac signal by means of the multiparameter fit described below.

Experiment 3 is a T_2 determination of the entire perfused heart system and uses a Hahn spin echo pulse sequence. No 180* inverting pulse is applied before the Hahn echo sequence, but note that this is equivalent to using the IHE pulse sequence with an ID value of infinity. The purpose of experiment 3 is to provide information about the relative cardiac and perfusate magnitudes. This is possible due to the extreme difference in T_2 relaxation times between the cardiac T_2 decay emphasized in experiment 1 and the perfusate T_2 decay.

Data analysis was performed by fitting integrals of the resonances to the equations described below. A gradient expansion algorithm (16), implemented in Basic on a personal computer, was used for curve fitting. This allowed flexibility in specifying the nonlinear equations which needed to be fit.

The equation which describes the data from an IHE pulse sequence for a compartment described by one T_1 value and one T_2 value, such as the perfusate, is:

$$M_p = M_{op}(1 - Fe^{-ID/a})\, e^{-TE/b}$$

where F represents [1 − cos(alpha)] in which alpha is the precise flip angle from the initial 180* pulse, a is the T_1 value, b is the T_2 value, and M_{op} is the total magnitude of the perfusate signal. ID and TE are the independent delay times defined earlier.

From visual inspection of the data from experiment 1, it was found that two time constants were needed to specify the T_2 decay of the cardiac signal. Cardiac T_1, in accord with previous findings for intracellular sodium and sodium in agarose gels, is expected to be adequately characterized by a single T_1 value. The equation which describes the data from an IHE pulse sequence for the cardiac component is then:

$$M_i = M_{oi}(1 - Fe^{-ID/c})(De^{-TE/d} + Ee^{-TE/e}),$$

where F is the same flip effect as before, c is the T_1 value, and D and E are the fractions of signals present at T_2 values d and e, respectively. The equation used to fit data from the entire perfused heart system, which includes both perfusate and cardiac contributions, is then the sum of the two equations, $M = M_p + M_i$.

The value of perfusate T_1 (parameter a) is approximately known and can be fixed before performing the fit. Perfusate T_1 was estimated from the temperature in the room and a calibration curve between perfusate T_1 and temperature. The uncertainty in this estimate of perfusate T_1 was judged to be ±2 ms, and this uncertainty was included in the analysis by performing the data analysis with the perfusate T_1 at its minimum and maximum possible values. Perfusate T_2 was not fixed in the analysis because the flow of perfusate affected the T_2 decay, making it difficult to quantify the actual T_2 time constant. Therefore, the equation $M = M_p + M_i$ contains eight free parameters to be specified by 56 total data points obtained from experiments 1–3.

As stated earlier, the signal referred to as cardiac actually contains a contribution from interstitial and intracellular ions. To determine the relaxation characteristics of interstitial ions, the intracellular sodium relaxation times and signal magnitude must be known. Intracellular relaxation times have been previously measured in perfused frog hearts using the addition of a shift reagent to the buffer (6). The effect of doubling the concentration of shift reagent on the intracellular relaxation times was found to be negligible, indicating that the intracellular relaxation times are probably not altered due to susceptibility effects in perfused heart preparations (6). Because the intracellular relaxation times of rat hearts had not yet been determined, they were measured in four hearts in separate experiments.

After NMR experiments utilizing the IHE pulse sequence, which were performed during perfusion with a normal buffer, the magnitude of the intracellular sodium signal was determined by perfusing several hearts with a buffer containing shift reagent. Based on literature estimates of compartmental volumes and concentrations in frog (17–19) or rat (20) hearts, the intracellular contribution is expected to be at most 20% of the total heart magnitude for both frog and rat. Comparison of the intracellular magnitude obtained using shift reagent with the combined interstitial and intracellular signal obtained by the multiparameter fit described above revealed that the fraction actually measured for intracellular contribution was 8% (frog) or 22% (rat) (see Results).

Knowing the intracellular magnitude and relaxation times, the intracellular contribution to the combined interstitial and intracellular measurements was subtracted to yield interstitial relaxation times. Comparison of these purely interstitial relaxation times with relaxation measurements of the combined interstitial and intracellular compartments revealed differences of <15% in the relaxation times. This is due to the fact that the intracellular signal was found to have a magnitude which was a small fraction of the magnitude of the combined intracellular and interstitial signal and had relaxation times similar to those of the two compartments combined (see Results). Therefore, the majority of the hearts did not receive shift reagent in the buffer due to the small error incurred by not correcting for intracellular signal, the time required for the shift reagent to completely wash in and out of the heart, and possible physiological effects of the shift reagent on measurements performed subsequent to addition of the shift reagent, such as the

blotted heart measurements. Differences in these relaxation times from those of pure interstitium of 15% or less were deemed of minor importance due to the fact that the goal of these interstitial relaxation time measurements was not to obtain a high precision, but to report for the first time the general characteristics of interstitial sodium relaxation.

Finally, NMR measurements of blotted heart tissue were performed. Magnitude, T_1, and T_2 measurements were done on this nonperfused heart tissue, with magnitude measurements being relative to the previous cardiac experiments. T_1 measurements used an inversion recovery pulse sequence, whereas T_2 measurements used a Hahn spin echo pulse sequence. T_1 data from nonperfused heart tissue were fit to a single exponential recovery curve, whereas T_2 data were fit to a biexponential decay curve.

RESULTS

Phantoms

The T_1 and T_2 values for sodium in both Ringers and Krebs Henseleit buffer were found to be equal with a value of 56 ms at room temperature. With small variations in room temperature of 24 ± 2°C, this relaxation time was found to vary by about ±1.5 ms. The T_1 relaxation time of the phantom with Ringers flowing at a rate comparable to that in the perfused heart preparation was found to be the same as the T_1 with no flow. However, the T_2 of this phantom was found to be 15 ms shorter, presumably due to sodium movement within B_0 inhomogeneities. The actual T_2 in the perfused heart preparations can be shorter than this due to the additional movement of the heart. Up to echo times of 100 ms, this T_2 decay was accurately characterized by a single exponential process. At greater echo times, the signal dropped off more rapidly than an exponential process would dictate, but this was not a problem as the maximum echo time used for T_2 measurements was 90 ms.

The IHE pulse sequence was applied to a phantom of 150 mM NaCl to determine if the pulse sequence produced artifactual signals or relaxation times. With *ID* fixed to values near the value which would completely cancel the sodium signal, the T_2 decay was measured and found to agree with the T_2 value obtained using a Hahn spin echo sequence. With *TE* fixed to a short value (0.32 ms), *ID* was varied to obtain a T_1 recovery curve, and the T_1 value obtained agreed with that found from an inversion recovery pulse sequence. Therefore, the IHE pulse sequence does not appear to produce artifacts in relaxation times.

The agarose, when studied separately, was found to have sodium relaxation times of T_1 = 38.5 ± 0.3 ms (mean ± SD), and T_2 = 5.4 ± 0.3 ms (60%) and 36.2 ± 2.3 ms (40%). These values were determined by an inversion recovery pulse sequence for T_1 and by a Hahn spin echo sequence for T_2. When the IHE pulse sequence and eight parameter fit was applied to the agarose + NaCl phantom, the relaxation times of agarose and the relative magnitudes of signal from NaCl solution and agarose agreed with those found in the separate experiments. The maximum difference in relaxation times between these values and the separately determined agarose values was 5%, whereas the error in the estimate of the magnitude of agarose signal was at most 20%. The *ID* value which resulted in nulling of the signal from the NaCl solution was set in the same fashion for this phantom as it was for the perfused hearts, namely by monitoring the signal at long echo times while trying different *ID* values.

Frog and rat hearts

Frog hearts perfused with Ringers solution beat steadily for up to 24 h. Rat hearts perfused with Krebs Henseleit buffer beat steadily for 5–8 h. The total duration of the experiment was ~4 h.

Cardiac relaxation times were measured for six frog hearts during perfusion with Ringers. For rat hearts, five cardiac relaxation time measurements were performed on three rat hearts (two hearts were measured twice) during perfusion with Krebs Henseleit. These results are summarized in Table 1 and are expressed as mean ± SD. T_1 data were fit with a single exponential time constant, whereas T_2 data had a biexponential nature and were therefore fit with two time constants, designated fast (f) and slow (s) with relative amplitudes of $\%T_{2f}$ and $\%T_{2s}$. The data was also analyzed assuming possible variations in perfusate T_1 as discussed earlier. The possible range of the mean cardiac T_1 was found to be 29.1–33.6 ms for frog hearts and 24.3–29.9 ms for rat hearts. The possible range of the mean short T_2 values was found to be 1.8–2.1 ms for frog hearts and 2.0–2.2 ms for rat hearts, whereas for the long T_2 values the range was 21.0–26.6 for frog hearts and 20.1–28.2 ms for rat hearts.

Table 2 lists the small effect intracellular sodium signal has on combined interstitial and intracellular relaxation

TABLE 1 Cardiac sodium relaxation times in perfused hearts

Heart	*n*	T_1	$\%T_{2f}$	T_{2f}	$\%T_{2s}$	T_{2s}
		ms		*ms*		*ms*
Frog	6	31.6 ± 3.0	37.2 ± 7.4	1.9 ± 1.0	62.8 ± 7.4	25.2 ± 1.3
Rat	5	27.3 ± 1.6	42.2 ± 4.7	2.1 ± 0.3	57.8 ± 4.7	26.3 ± 3.2

TABLE 2 **Comparison of magnitudes and relaxation times of total cardiac, intracellular, and interstitial compartments**

	Relative magnitude	T_1	$\%T_{2f}$	T_{2f}	$\%T_{2s}$	T_{2s}
	%	*ms*		*ms*		*ms*
Frog hearts ($n = 3$)						
Cardiac*	100	29.4 ± 3.9	41.6 ± 7.3	1.8 ± 1.0	58.4 ± 7.3	23.8 ± 0.9
Intracellular‡	8.4 ± 4.9	22.4 ± 3.0	48.0 ± 8.0	2.0 ± 1.3	52.0 ± 8.0	16.4 ± 4.2
Interstitial‡	93.4 ± 6.1	30.7 ± 4.4	40.3 ± 8.6	1.8 ± 1.1	59.7 ± 8.6	24.5 ± 0.8
Rat hearts ($n = 2$)						
Cardiac*	100	31.0 ± 1.5	28.4 ± 18.4	1.7 ± 1.3	71.6 ± 18.4	22.5 ± 6.0
Intracellular‡	21.7 ± 9.3	23.0 ± 2.5	23.8 ± 6.0	2.6 ± 0.8	76.2 ± 6.0	19.0 ± 1.1
Interstitial‡	81.9 ± 1.4	34.2 ± 4.9	31.1 ± 20.3	1.5 ± 1.5	68.9 ± 20.3	25.0 ± 7.4

*Cardiac refers to a combination of interstitial plus intracellular compartments.
‡Intracellular and interstitial magnitudes are relative to combined magnitude. They are derived from fits to differing data sets and add to 100% within error margin.

times of frog hearts. The cardiac relaxation times were obtained from the eight-parameter fit of the raw data. The intracellular magnitude was obtained by using shift reagent, whereas the intracellular relaxation times were characterized previously in frog hearts (6) and were measured in separate experiments on four hearts for rats. The purely interstitial relaxation times were obtained by the eight-parameter fit of the data after the intracellular contribution had been subtracted from the raw data. These values illustrate the validity of using the combined interstitial and intracellular sodium signal as a good estimate of interstitial relaxation times.

A typical interstitial T_2 decay curve obtained from a frog heart using the IHE sequence with the perfusate signal nulled (described as experiment No. 1 above) is shown in Fig. 2. The intracellular contribution, as determined by shift reagent experiments, has been subtracted from this curve to illustrate the fact that purely interstitial signal has a distinct biexponential behavior. The biexponential character of the interstitial decay, as seen by its nonlinear character on the semilogarithmic plot, was apparent for every heart used in the experiments.

It should be noted that upon perfusion with the shift reagent buffer, both the rate and force of contraction decreased, presumably due to the chelating of free calcium by the shift reagent. For frog hearts, the duration over which the beat was regular and detectable was not appreciably decreased by perfusion with shift reagent. For rat hearts the force of contraction decreased to ~10% of normal, and the duration over which its beat was detectable was reduced to ~2 h. Experiments with shift reagent required 1 h.

The relaxation times of nonperfused heart tissue are presented in Table 3. These values changed by <10% over 21 h of observation for frog hearts and by <20% over 21 h of observation for rat hearts. The direction of the changes was consistently toward shorter relaxation times. The magnitude of signal obtained from this preparation is also of interest. For frog hearts, the magnitude of the signal from nonperfused hearts was 1.9 ± 0.4 ($n = 3$) times as large as the cardiac signal estimated from perfused hearts, whereas for rats the nonperfused magnitude was 2.8 ± 0.5 ($n = 3$) times as large as the cardiac signal estimated from perfused hearts. Possible interpretations of this will be discussed below. The magnitude of this nonperfused tissue signal was constant from 3 min to 21 h after cessation of perfusion.

DISCUSSION

The techniques used to observe the cardiac sodium relaxation rely on differences in the relaxation times between sodium in the perfusate and sodium in the heart. Therefore, if the cardiac signal, or a component of the

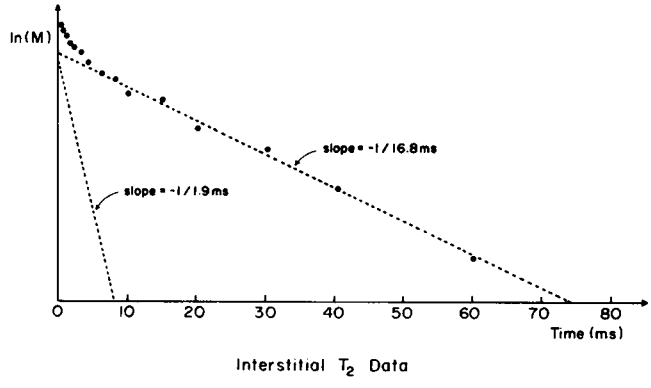

FIGURE 2 T_2 relaxation curve for interstitial sodium of frog heart using the IHE pulse sequence. As described in the text, intracellular sodium as measured with shift reagent has been subtracted from the cardiac signal. The magnitude of the subtracted intracellular signal was equal to 10% of the cardiac magnitude. The T_2 time constants of the subtracted signal were 2 ms (50%) and 16 ms (50%) (6).

TABLE 3 **Sodium relaxation times of nonperfused heart tissue**

Heart	n	T_1	$\%T_{2f}$	T_{2f}	$\%T_{2s}$	T_{2s}
		ms		*ms*		*ms*
Frog	3	42.4 ± 1.6	38.3 ± 4.9	3.3 ± 0.7	61.7 ± 4.9	36.3 ± 4.1
Rat	3	43.4 ± 4.9	26.7 ± 4.0	3.4 ± 0.6	73.3 ± 4.0	39.6 ± 5.0

cardiac signal, has relaxation times similar to those of the perfusate, then these methods will not be able to observe and quantify this signal. Thus, in characterizing the cardiac signal in this way, it is important to realize that only cardiac components which have shorter relaxation times than the perfusate have been studied; cardiac signal with T_1 and T_2 values similar to those of the perfusate will be missed or underrepresented with these techniques.

One possible explanation as to why the magnitude of the signal from nonperfused heart tissue is greater than the cardiac magnitude determined from perfused preparations is that the NMR techniques used here have detected only a component of the signal from the heart. The fact that nonperfused relaxation times are somewhat longer than the corresponding perfused cardiac relaxation times is in accordance with this possibility.

Similarly, only the cardiac sodium which is NMR visible under these conditions has been studied. Because specific visibility experiments have not been conducted on cardiac sodium, the possibility of invisible sodium, namely sodium whose T_2 value is so short as to be undetectable with the parameters of this study, still exists.

Concerning the sodium that is observed, the data is fit to equations of a single T_1 time constant and two T_2 time constants because this adequately characterizes the data. Less than two T_2 time constants would not have provided a good fit, and more time constants would not be justified for the available signal-to-noise ratio. Note that this provides merely a characterization of the cardiac relaxation and not a description of the distribution of time constants which may be physically present. The interstitium, which provides the majority of the cardiac signal, is a heterogeneous space with features that may produce a multitude of NMR interactions for the sodium nuclei.

With these facts in mind, the cardiac relaxation times found here can be interpreted and applied to further spectroscopy and imaging studies. Given the time constants measured for the cardiac signal and the small effect of the intracellular ions, the primary finding is that a component of the interstitium possesses short relaxation times for perfused hearts under control conditions. This includes a biexponential T_2 decay with one component of ~2 ms. Thus the interstitium contains sodium relaxation times which are similar to the relaxation times found for the intracellular sodium of perfused hearts. This indicates that it will be difficult to separate the intracellular and extracellular sodium signal on the basis of relaxation times under control conditions. Methods which rely, either directly or indirectly, on differences in relaxation times probably will not work.

Attempts to separate intracellular and extracellular signals on the basis of relaxation times still have the potential to provide useful information in pathological tissue. It is possible that one or both compartments will have altered sodium relaxation times under pathological conditions enabling the detection of the pathological tissue and the monitoring of the sodium signal arising from each compartment. Studies on excised human colon and breast tissue have revealed that a substantial amount of short T_2 signal is present in both normal and cancerous tissue (21). However, contrast between normal and pathological tissue has been shown to exist on sodium images with variable echo times, indicating that interstitial relaxation times may be changed under certain pathological conditions such as gross edema (5).

Further studies on extracellular sodium relaxation were performed on cartilage tissue. Cartilage is a nearly acellular tissue in which parameters known to affect the extracellular space, such as fixed charge density and hydration, can be easily varied and measured. Preliminary results indicate that the T_1 of interstitial sodium in cartilage at physiologic pH is ~20 ms while the T_2 is biexponential with time constants of ~1.5 ms (65%) and 18 ms (35%). These values change with large changes in fixed charge density and hydration; however, in all cases there exists a component of T_2 decay with a time constant between 0.7 ms and 9 ms, with the longer time constants only occurring when the pH value is below 2.5 (22). This further substantiates the finding of a short T_2 component in an interstitial space.

The finding of short relaxation times in the interstitium increases the difficulty of separating the sodium NMR signal originating from the intracellular and extracellular spaces. Possibilities still exist, however, for obtaining this valuable compartmental information using NMR. Multiple quantum spectroscopy with paramagnetic quenching of the extracellular signal (23), and future development of a nontoxic shift reagent with uniform in vivo distribution remain possibilities. This work has been performed only on hearts under control conditions, and the interstitial relaxation times for various pathological states may be different. In particular, the sodium relaxation characteris-

tics of edematous tissue, infarcted tissue, and tumors should be studied. Such studies may lead to an increased understanding of ion compartmentation in these disease states, as well as to an increased ability to detect and delineate regions of pathology in sodium NMR images.

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