Evaluation of Triple-Quantum-Filtered ^{23}Na NMR in Monitoring of Intracellular Na Content in the Perfused Rat Heart: Comparison of Intra- and Extracellular Transverse Relaxation and Spectral Amplitudes

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Multiple-quantum filtered (MQF) NMR offers the possibility of monitoring intracellular (IC) Na content in the absence of shift reagents (SR), provided that (i) the contribution from IC Na to the MQF spectrum is substantial and responds to a change in IC Na content, and (ii) the amplitude of the extracellular (EC) MQF component remains constant during a change in IC Na content. The validity and basis for these conditions were examined in isolated perfused rat hearts using SR-aided and SR-free triple-quantum filtered (TQF) ^{23}NaNMR. Despite a myocardial Na content that was only ~1/70 that of EC Na. IC Na contributed to over 25% of the total TQF spectrum acquired in the absence of SR. Transverse relaxation times (T_2) were approximately twice as long for EC compared to IC Na, despite SR-induced relaxation of T_2 for the former pool. However, the efficiency of generation of the TQF signal was similar for IC and EC Na, indicating that a much greater percentage of IC relative to EC Na exhibits TQ coherence. During constant perfusion with ouabain (0.2 mM for 25 min) or with a hypoxic and aglycemic solution (50 min), the amplitude of the IC TQF spectrum increased by ~330% and ~280%, respectively. In contrast, the amplitude of the EC TQF spectra remained essentially constant for both interventions. The amplitude for IC Na increased ~250% relative to baseline during no-flow ischemia (60 min), whereas the amplitude of the EC TQF spectra decreased by ~33% before stabilizing. In SR-free experiments, the TQF spectral amplitude increased ~2-fold during the constant perfusion interventions, but did not change significantly during no-flow ischemia. These data suggest that the change in the TQF spectral amplitude during constant perfusion interventions is from IC Na, and that TQF techniques in the absence of SR may be useful in monitoring IC Na during these interventions. The fall in the amplitude of the EC TQF spectral amplitude during no-flow ischemia complicates the use of TQF techniques without SR during this intervention.

Key words: sodium; NMR; multiple-quantum filters; perfused hearts.

INTRODUCTION

Intracellular (IC) sodium (Na) is involved in multiple cellular activities in the myocardium, including electrophysiologic processes, membrane transport, muscle contraction, and gene expression (1). Availability of improved techniques to monitor IC Na in cardiac tissue will, therefore, help advance understanding in many spheres of cardiac physiology. Ion-selective microelectrodes, electron microprobe x-ray analysis, fluorescent dyes, and whole tissue analysis (2, 3) can be used to measure IC Na content but require tissue disruption and cannot be applied to intact beating hearts.

^{23}Na NMR spectroscopy allows tissue Na content to be monitored noninvasively in both cell preparations and whole organ systems (4–8). Because the Larmor frequency is identical for IC and extracellular (EC) Na, a paramagnetic shift reagent (SR) is required to resolve these two components of the ^{23}Na spectrum. However, SRs have disadvantages, including inadequate resolution of the IC and EC resonances and chelation of divalent cations, leading to depression of myocardial contractility (4, 7, 9). Although the latter problem can be overcome by increasing perfusate calcium concentration, the cost of SRs and their perturbation of the chemical milieu make their use less than ideal for studying physiological processes in the heart.

In principle, multiple-quantum-filtered (MQF) ^{23}Na NMR spectroscopy may provide increased sensitivity in detecting IC Na and thus offers the possibility of monitoring changes in IC Na content without SR. In contrast to single-quantum (SQ) NMR, in which the area of the SQ spectrum is proportional to Na content alone (neglecting any NMR-invisible fraction), the amplitude of the MQF spectrum is determined by several factors. These include transverse relaxation times, the creation time used in the MQF pulse sequence, and the amount of Na that exhibits MQ coherence (10–12; also, note that the SQ spectral amplitude, as opposed to the area, is dependent on transverse relaxation times). If either the efficiency of generation of the MQF signal (as determined by relaxation behavior) or the percentage of Na exhibiting MQ coherence is greater for IC versus EC Na, then IC Na will make a contribution to the MQF spectrum that is disproportionately greater than the ratio of IC:EC Na content in the NMR chamber. Data in perfused hearts and other biological models demonstrate a disproportionately increased contribution from IC Na to the MQF spectrum and suggest increased sensitivity of MQF versus SQ techniques in monitoring IC Na content (11, 13, 14–17). However, the fundamental basis for the increased sensitivity of MQF NMR for IC Na and, hence, the validity of MQF techniques without SR, is unclear. Knowledge of IC and

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EC transverse relaxation times is required to compare the efficiency of generation of the IC versus EC Na MQF signal. Only indirect data concerning EC Na transverse relaxation times exists (18, 19).

In addition to a substantial signal from IC Na, the contribution from EC Na to MQF spectra acquired in the absence of SR must remain constant or quantifiable, so that a change in the amplitude of the total MQF spectrum can accurately reflect a change in IC Na content. This is true because the contribution to the MQF spectrum from EC Na has been shown to be significant under normal conditions, and, therefore, must be accounted for (11, 16, 20, 21). However, only indirect data exists as to the behavior of the EC MQF resonance during interventions (19).

Therefore, the purpose of this study was twofold: 1) to measure directly and compare transverse relaxation times for IC and EC Na and 2) to monitor directly the IC and EC contributions to the MQF spectrum during physiological interventions in isolated perfused hearts. SR-aided and SR-free triple-quantum filtered (TQF) ^{23}Na NMR spectra were acquired at baseline and during interventions designed to increase IC Na content (constant perfusion of ouabain or a hypoxic-aglycemic solution, as well as no-flow ischemia). The results indicate that IC Na makes a contribution to the total TQF spectrum that is disproportionately greater than the ratio of IC:EC Na content, due to a greater relative proportion of IC Na exhibiting TQ coherence, not because of more efficient generation of the IC TQF signal. In addition, this study demonstrates that the EC TQF signal remains stable during constant flow interventions that increase the IC TQF signal. These results suggest that TQF Na NMR may be useful in monitoring IC Na without SR in perfused rat hearts during constant perfusion interventions.

METHODS

Heart Perfusion

Male Wistar rats, weighing approximately 400 g, were anesthetized with ketamine (60 mg/kg) and xylazine (20 mg/kg) and given 1000 units intraperitoneal heparin before surgery. After complete anesthesia, the sternum was incised, the pericardium was removed, and the heart was excised above the great vessels. The heart was then immediately perfused in a retrograde manner at a constant perfusion pressure of 90 mmHg and submerged in perfusate within a 20-mm glass NMR tube. A suction tube placed above the heart collected perfusate effluent. Left ventricular pressure was monitored via a balloon placed within the left ventricle, with the balloon volume adjusted to an end-diastolic pressure of 10–12 mmHg and not changed for the duration of the experiment.

Perfusate and Shift Reagent

Perfusate used for the heart preparation included a modified Tyrode's solution (NaCl 144 m*M*, KCl 5 m*M*, $MgCl_2$ 0.9 m*M*, Hepes 6 m*M*, $CaCl_2$ 1.5 m*M*, dextrose 15 m*M*) or a modified Krebs-Henseleit solution (NaCl 118 m*M*, KCl 6 m*M*, $CaCl_2$ 1.5 m*M*, $MgSO_4$ 1.2 m*M*, Na_2EDTA 0.5 m*M*, $NaHCO_3$ 25 m*M*, and dextrose 15 m*M*). All perfusate solutions were adjusted to pH 7.4, heated to 35°C, and bubbled with 95% O_2:5%N_2 (Hepes) or 95% O_2:5% CO_2 (Krebs-Henseleit). Thulium (III) complex of 1,4,7,10-tetraazacyclododecane - N,N′,N″,N‴tetra(methylenephosphonate)[Tm (DOTP)$^{5-}$] was the shift reagent used to resolve IC and EC Na NMR spectra (supplied by C. Malloy and A. D. Sherry, University of Texas, Dallas). This reagent provides adequate chemical shift and less cardiotoxicity than the SRs, Dy $(PPP)_2^{7-}$ and Dy $(TTHA)_2^{7-}$ (9). Due to the direct interaction of Tm (DOTP)$^{5-}$ with EC Na, attenuation of the transverse relaxation times and spectral amplitudes for EC Na was anticipated. To minimize these effects, the lowest concentration of Tm (DOTP)$^{5-}$ required to provide adequate resolution of spectra was used (4.5 m*M*). The concentration of Ca^{2+} in the perfusate was increased by 3.0 m*M* (to a final concentration of 4.5 m*M*) to maintain a free Ca^{2+} level of about 1 m*M*, which was confirmed by a Ca^{2+} sensitive electrode (Orion). The left ventricular developed pressure remained constant for at least 2 h after addition of SR to the perfusate.

NMR Methods

All NMR experiments were performed with a 20-mm ^{23}Na probe on a Bruker WB-AM 300 spectrometer, equipped with an Aspect 3000 pulse programmer. TQF spectra were acquired at 79.4 MHz, using 4000 data points, a sweep width of 4 KHz, and 384 transients (3.5-min acquisition time). TQF spectra were acquired using the pulse sequence:

$$90^\circ_{\phi_1} - \frac{\tau}{2} - 180^\circ_{\phi_2} - \frac{\tau}{2} - 90^\circ_{\phi_3} - \delta - 90^\circ_{\phi_4} - \mathrm{acq}_{\phi_5}(t), \qquad [1]$$

where ϕ_1 is the phase value, τ is the creation time, δ is the evolution time (20 μs), t is the acquisition time (500 ms), and a 192-step phase cycling scheme was used to select for TQ coherences (11). Amplitudes of TQF ^{23}Na NMR spectra were measured from baseline to peak (the integral of the MQF spectrum is equal to 0, thus spectral amplitudes were used). In all cases, free induction decays were exponentially multiplied with a line broadening of 10 Hz before Fourier transformation. An experiment in a perfused heart, examining the modulation of TQF spectral amplitudes when the carrier frequency was positioned off-resonance from the IC and EC peaks, confirmed that the TQF signals contained no contributions from SQ coherences (20). An additional experiment in a heart that was not submerged in perfusate demonstrated no change in the IC/EC ratio of TQF spectral amplitudes, when compared with the submerged heart, indicating no contribution from the perfusate bath to the EC TQF signal.

The Fourier transformed form of the TQF net magnetization is as follows (10):

$$M_M(\omega, \tau, \mathrm{Na}) = \frac{9}{40} M_o(\mathrm{Na}) \left(e^{-\frac{\tau}{T_{2s}}} - e^{-\frac{\tau}{T_{2f}}}\right) \cdot \left(\frac{\frac{1}{T_{2s}^*}}{\left(\frac{1}{T_{2s}^*}\right)^2 + \omega^2} - \frac{\frac{1}{T_{2f}^*}}{\left(\frac{1}{T_{2f}^*}\right)^2 + \omega^2} \right), \qquad [2]$$

where M (ω, τ, Na) is the TQF magnetization, M_o(Na) is the equilibrium magnetization, T_{2s} and T_{2f} denote the slow and fast transverse relaxation times, respectively, T_{2s}* and T_{2f}* denote the corresponding inhomogeneous broadened forms, and ω is the resonance offset. Transverse relaxation times were determined from TQF spectra acquired from a series of creation times, varying from 0.2 to 70 ms, by fitting the relation between spectral amplitude and τ to Eq. [2] (spectra acquired on resonance, $\omega = 0$). A modified Marquardt algorithm was used to achieve the best statistical fit to the data (22).

Protocols

The first experiment was designed to estimate the effect of SR on EC Na spectral amplitudes. This was done by acquiring TQF spectra for different τ in a perfused heart both before and after exposure to SR and analyzing the IC and EC spectral amplitudes.

In the next series of experiments ($n = 7$), hearts were perfused with solution containing SR, and TQF spectra were acquired for various τ values to determine IC and EC transverse relaxation times.

Finally, three interventions were used to affect myocardial IC Na content: 1) ouabain (0.2 m*M*), a Na-K pump inhibitor, was added to the perfusate ($n = 3$); 2) combined hypoxia and aglycemia were induced by changing the perfusate from base-line oxygenated solution containing dextrose to a dextrose-free perfusate that was preboiled to remove O_2 and bubbled with N_2 during the experiment ($n = 3$); and 3) no-flow ischemia was induced by completely interrupting perfusate flow ($n = 3$). Each heart studied was subjected to only one of these interventions. TQF spectra in these hearts were acquired at the τ value that maximized the IC Na spectral amplitude (τ_{max}) under base-line conditions (4 ms). The IC and EC spectral amplitudes during intervention were normalized to the preceding base-line spectra. Identical experiments were performed in the absence of SR ($n = 3$ for each intervention). Results for each intervention were summarized by presenting mean (± SD) changes in normalized spectral amplitude as a function of time.

RESULTS

Attenuation of EC Spectral Amplitudes by SR

The degree of attenuation of the EC Na spectra by SR was estimated by comparing the SR-attenuated amplitudes of EC TQF spectra with SR-free amplitudes of the EC TQF spectra (Fig. 1). The latter were estimated by subtraction of the IC Na spectral amplitude, determined in the presence of SR, from the amplitude of the TQF spectra acquired in the absence of SR, for each τ. The degree of attenuation of the calculated amplitudes for the EC spectra depended on the creation time, because the calculated amplitudes for spectra acquired at short τ values were attenuated by at least 50%, and the attenuation was significantly larger at longer τ values (Table 1). The τ_{max} for the EC shifted spectra (~12 ms) was half the value of τ_{max} for the calculated SR-free EC spectra (~24 ms). At τ_{max} for the IC TQF component (~4 ms), the spectral amplitude from IC Na comprised over 25% of the total amplitude of the TQF spectrum.

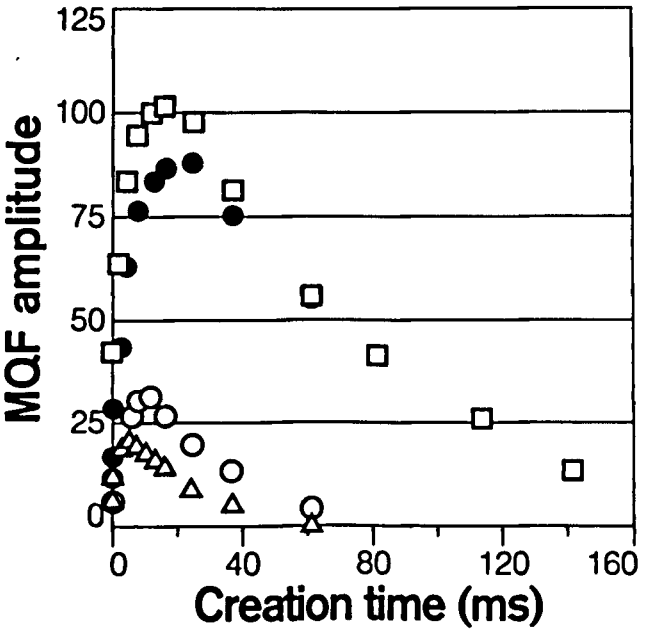

FIG. 1. A plot of amplitude of TQF ^{23}Na NMR spectra for an isolated perfused rat heart versus creation time for the total TQF spectrum (open squares) acquired in the absence of SR, IC TQF (open triangles), and EC TQF (open circles) spectra acquired in the presence of SR, and corrected amplitudes for the EC TQF spectrum (closed circles), calculated from the difference in amplitudes between total TQF and IC TQF spectra. The percentage attenuation of the TQF spectra was calculated using the amplitudes plotted here (see Table 1). At longer τ (> 60 ms), the IC TQF spectral amplitude approaches 0, so that the corrected EC TQF spectral amplitude is equal to the total TQF spectral amplitude. τ_{max} for the observed and calculated EC spectra were ~12 and ~24 ms, respectively.

Table 1
Attenuation of the Extracellular TQF Spectra by Tm (DOTP)$^{5-}$

τ (ms)	% Attenuation of extracellular TQF signal
0.4	55.0 ± 16.8
0.8	57.5 ± 8.7
2.0	64.2 ± 9.2
4.0	62.6 ± 8.4
8.0	64.7 ± 9.4
12.0	67.8 ± 6.0
16.0	70.5 ± 2.8
24.0	76.8 ± 4.6
36.0	83.4 ± 4.0
60.0	89.3 ± 8.2
80.0	100.0 ± 0.0

Transverse Relaxation Times for Intra- and EC Na Ion Pools

Representative plots of IC and EC TQF spectral amplitudes versus τ are shown in Fig. 2. The fitting of the TQF spectral amplitudes for EC Na to Eq. [2] was generally poorer than that for IC Na. The calculated transverse relaxation times, T_{2s} and T_{2f}, are shown in Table 2 ($n = 7$). T_{2s} was significantly longer than T_{2f} for IC Na (17.0

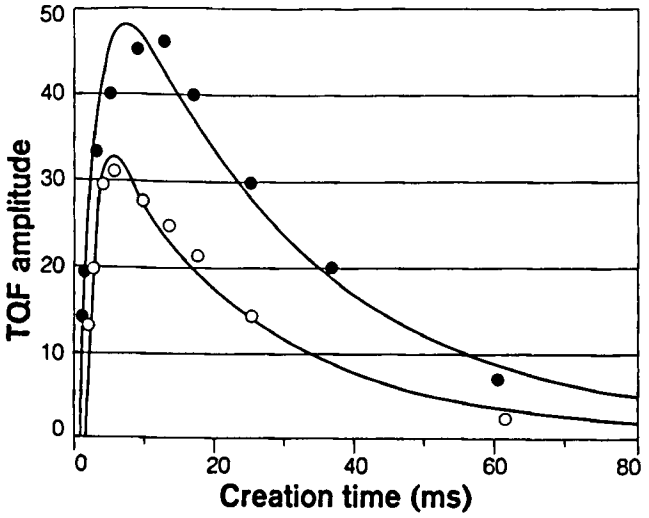

FIG. 2. A representative plot of the amplitude of IC (open circles) and EC (closed circles) TQF spectra versus creation time, τ. The amplitudes for both the IC and EC TQF spectra were best fit by biexponential functions; τ_{max} for the IC and EC spectra were ~4 and ~12 ms, respectively.

Table 2
NMR Transverse Relaxation Times Calculated for Intra- and Extracellular Na

	Creation time experiments	
	Intracellular Na	
T_2f	1.0 ± 0.2	n = 7
T_2s	17.0 ± 3.3	
	Extracellular Na	
T_2f	2.2 ± 0.3	n = 7
T_2s	29.3 ± 5.3	

Values are represented in milliseconds ± SD, and n denotes the number of hearts examined.

versus 1.0 ms, respectively), as well as for EC Na (29.3 and 2.2 ms, respectively), reflecting biexponential relaxation for both IC and EC Na. Both T_{2s} and T_{2f} for IC Na were shorter than the corresponding values for EC Na, despite the SR-induced enhancement of EC Na relaxation. The creation time that maximized the TQF spectral amplitude was shorter for IC (τ_{max} = ~4 ms) than for the EC spectra (τ_{max} = ~12 ms) (Fig. 2).

Evaluation of Changes in Amplitude of IC and EC Triple-Quantum Filtered ^{23}Na NMR Spectra with Interventions

Isolated hearts were subjected to maneuvers known to alter IC Na content, and the amplitudes of IC and EC TQF Na spectra (acquired at τ = 4 ms to maximize the IC amplitude) were monitored. After allowing a 20-min equilibration period after addition of SR, several base-line spectra were acquired, and ouabain was added to the perfusate. Spectra from a representative experiment are shown in Fig. 3a, which shows three base-line spectra, followed by six spectra obtained at ~3.5-min intervals during 31 min of ouabain exposure (IC peaks are upfield from EC peaks in each pair of spectra). The addition of 0.2 m*M* ouabain resulted in a rapid and progressive increase in the amplitude of the IC TQF spectra (increasing by ~330%, on average, after 25 min of exposure, n = 3),

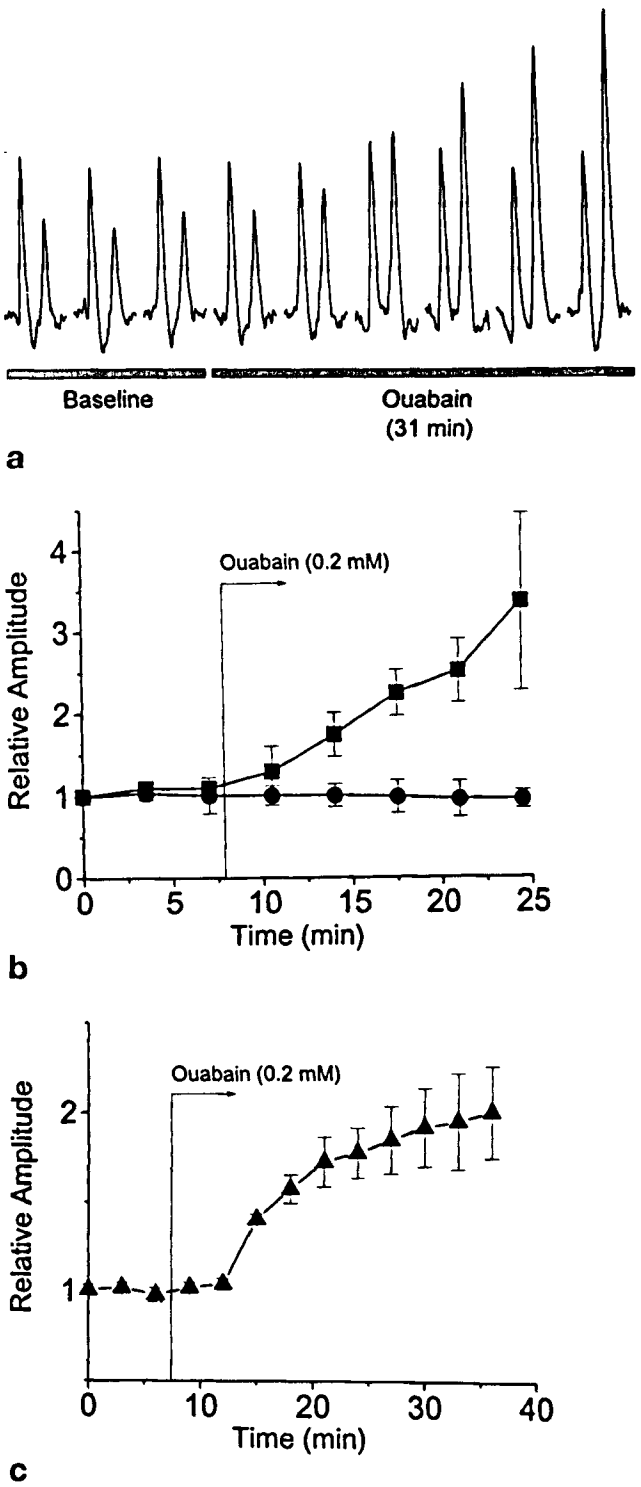

FIG. 3. (a) TQF spectra (τ = 4 ms) of a perfused rat heart acquired over a period of 31 min of perfusion with 0.2 m*M* ouabain. Each spectrum consists of two peaks. The resonance for IC Na is the upfield peak in each spectrum. (b) Plot of relative spectral amplitude versus time of exposure to ouabain (0.2 m*M*) for perfused hearts (n = 3). Data are normalized to base-line IC (squares) and EC (circles) spectra. Error bars represent SD. The IC spectra increased by ~330% while the EC spectra remained within ~20% of base-line levels. (c) Plot of relative spectral amplitude versus time of exposure to ouabain (0.2 m*M*) for hearts perfused in the absence of SR (n = 3). The spectral amplitudes increased by ~200%.

whereas the amplitude of the EC TQF spectra varied by less than ~20% of baseline (Fig. 3b, data normalized to base-line spectra). Identical experiments performed in the absence of SR (n = 3) demonstrated that the total TQF spectral amplitude increased ~200% after similar exposure to ouabain (Fig. 3c).

In the next group of experiments (n = 3), hypoxia and aglycemia were simultaneously induced after stabilization with normal perfusate. Representative spectra are shown in Fig. 4a. The IC Na spectral amplitude rose rapidly, reaching ~280% of baseline by 50 min, whereas the EC amplitude varied by less than ~10% of baseline for the duration of the experiment (Fig. 4b, normalized data). Experiments performed in the absence of SR (n = 3) demonstrated a ~160% increase in the total TQF spectral amplitude (Fig. 4c).

In a final group of hearts, after loading of SR and acquisition of base-line TQF Na NMR spectra, no-flow ischemia was induced by discontinuing perfusate flow (n = 3). Representative spectra are shown in Fig. 5a. Exposure to 60 minutes of no-flow ischemia resulted in a significant increase in the amplitude of the IC TQF spectra (mean ~250%), whereas the amplitude of the EC TQF spectra decreased immediately and then stabilized at ~66% of baseline after ~20 min of ischemia (Fig. 5b, normalized data). Experiments performed in the absence of SR (n = 3) showed no significant change in the total TQF spectral amplitude (Fig. 5c).

Thus, although the amplitudes of the IC TQF spectra increased in response to an expected increase in IC Na content for all of the interventions, the response of the EC Na spectra differed between the constant perfusion interventions and no-flow ischemia. The differences between the types of interventions were mirrored in the results of the experiments without SR.

DISCUSSION

In 1986, Pekar et al. (23) applied double-quantum-filtered (DQF) Na NMR spectroscopy to dog red blood cells suspended in solution with SR and observed a single Na resonance, suggesting that DQF signals arose solely from IC Na. It is now recognized that at the concentration of SR used in that study, the EC signal was likely quenched, and it is now generally accepted that there is a significant EC contribution to MQF spectra derived from a third-rank tensor in biological systems. In addition, one group of investigators has suggested that IC and EC transverse relaxation times are similar in perfused rat hearts, using SR-aided SQ NMR (18), thus implying that relaxation-based techniques such as MQF NMR cannot resolve IC from EC Na. The same group reported an increase in the amplitude of the EC Na spectra associated with the fast component of the transverse relaxation times during ischemia and edema formation in perfused rat hearts, suggesting that the EC MQF spectrum would increase under these conditions (19). These cumulative data have suggested that MQF NMR cannot be used to assess changes in IC Na without SR.

Nevertheless, multiple studies in several biological models suggest an improved ability of MQF techniques over SQ NMR to qualitatively monitor changes in IC Na (11, 13, 14–17). Given the small ratio of IC:EC Na content in the NMR chamber, these data indicate that MQF signal generation is disproportionately increased for IC relative to EC Na. Examination of Eq. [2] reveals that transverse relaxation times, the value of the creation time, and equilibrium magnetization all contribute to the TQF spectral amplitude. To determine the basis for increased sensitivity of MQF techniques to detect IC Na, and to validate the technique in the absence of SR, a detailed analysis of these factors is required. In addition, quantitative analysis of changes in IC Na by MQF techniques without SR requires that the EC MQF component remain constant or is quantifiable during interventions that change IC Na content. Direct determination of the behavior of the EC component of the MQF spectrum during physiological intervention has not been performed; the study noted above (19) relied on an indirect determination of changes in EC Na, using SQ methods.

We examined these issues by determining transverse relaxation times and intervention-induced changes in TQF spectra by directly monitoring IC and EC spectra, using the SR Tm $(DOTP)^{5-}$. Relative to other commonly used SRs [Dy $(PPP)_2^{7-}$ and Dy $(TTHA)_2^{7-}$], a lower concentration of this reagent was required for adequate chemical shift, allowing less attenuation of TQF spectral amplitudes [this has been attributed in part to the lower effective magnetic moment of Tm^{3+} relative to Dy^{3+} (9)]. We could then directly determine the T_{2s} and T_{2f} values for the IC and EC Na environments, as well as monitor changes in IC and EC spectral amplitudes with interventions.

Analysis of Attenuated EC Spectral Amplitudes by SR

Because an important part of the current study involved analysis of chemically shifted and attenuated EC spectra, the effect of the SR on EC Na relaxation was estimated by comparing the SR-attenuated amplitudes of EC Na TQF spectra with those calculated by subtraction of the amplitudes of IC Na spectra from SR-free TQF spectral amplitudes (for each τ; Fig. 1). From Table 1, it can be seen that the degree of attenuation of the EC spectrum depended on τ, being smallest at short τ and becoming progressively larger at longer τ. The significant attenuation of EC spectra, even at short τ, indicates significant EC Na relaxation caused by the SR.

IC Contribution to MQF Spectral Amplitude

An important observation from the analysis of the attenuated EC spectra is that at τ_{max} for IC Na (~4 ms), the IC spectral component comprised over 25% of the total TQF amplitude in the absence of SR, similar to data reported by Jelicks and Gupta in the rat heart (24). Because IC Na at baseline comprises ~1/70th of the total cardiac Na content (estimate based on relative IC and EC volumes and Na concentrations; surrounding perfusate bath not accounted for, because it does not contribute to the TQF signal), this observation indicates that the TQF IC spectral component is ~23-fold greater than the EC component, based on Na content (and by analogy, TQF NMR is ~23 times more sensitive than SQ NMR for IC Na).

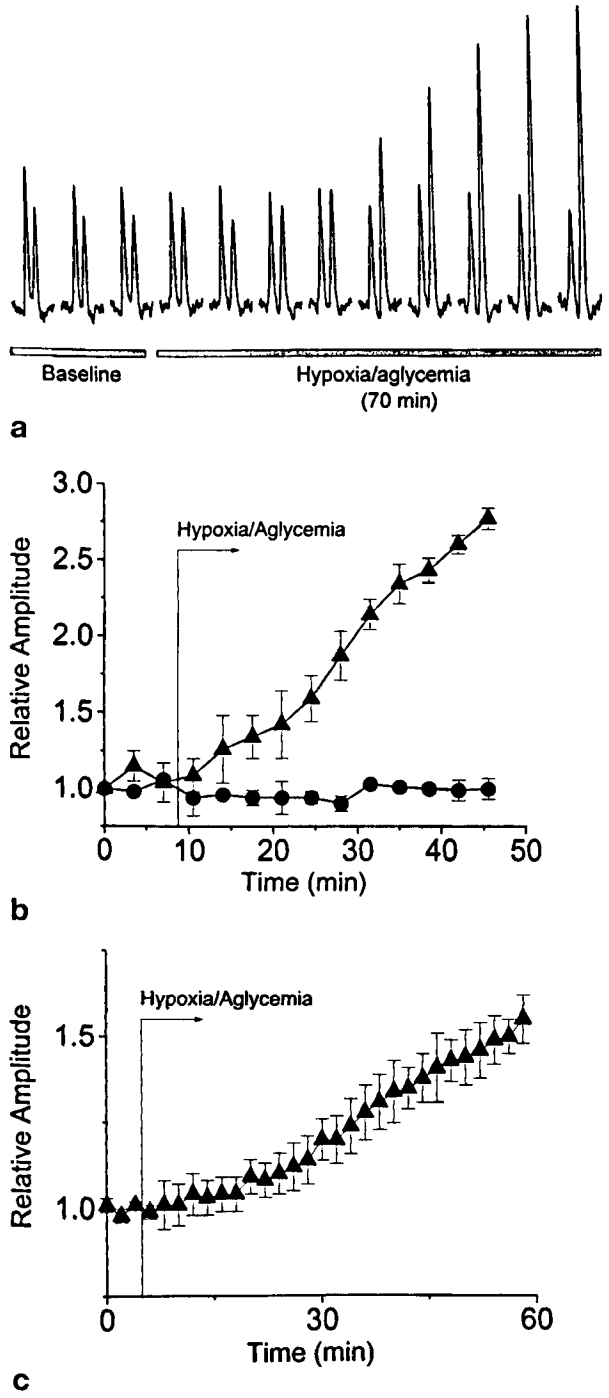

FIG. 4. (a) TQF spectra (τ = 4 ms) of a perfused rat heart acquired over a period of 70 min of hypoxia-aglycemia. (b) Plot of relative spectral amplitude versus time of exposure to hypoxia-aglycemia (n = 3). Data are normalized to base-line IC (triangles) and EC (circles) spectra. Error bars represent SD. The IC spectra increased ~280% while the EC spectra remained within ~10% of base-line levels. (c) Plot of relative spectrtal amplitude versus time of exposure to hypoxia-agllycemia for hearts perfused in the absence of SR (n = 3). The spectral amplitudes increased by ~160%.

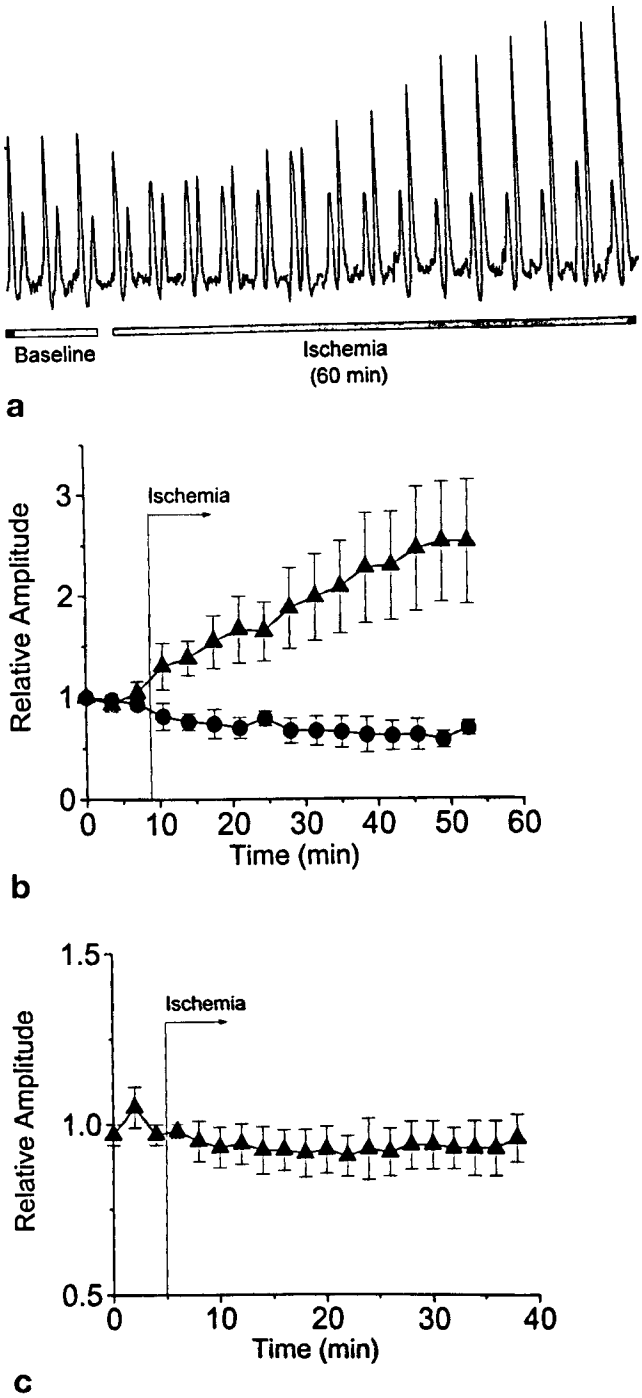

FIG. 5. (a) TQF spectra (τ = 4 ms) of a perfused rat heart acquired over a period of 60 min of no-flow ischemia. (b) Plot of relative spectral amplitude versus time of no-flow ischemia for perfused hearts (n = 3). Data are normalized to base-line IC (triangles) and EC (circles) spectra. Error bars represent SD. The IC spectra increased ~250% while the EC spectra initially decreased ~33% before stabilizing after ~20 min of ischemia. (c) Plot of relative spectral amplitude versus time of no-flow ischemia for hearts perfused in the absence of SR (n = 3). There was no significant change in the spectral amplitudes.

Relaxation Time Analysis

A comparison of transverse relaxation times for IC and EC Na ion pools allowed the differences in the IC and EC Na environments to be characterized. As was expected from the fact that MQF signals could be detected, T_{2s} was significantly longer than T_{2f} for both the IC and EC environments, reflecting biexponential relaxation (Table 2). Despite the enhanced relaxation induced by the SR for EC Na, the T_{2s} and T_{2f} times for EC Na were still approx-

imately twice those of their counterparts for IC Na. The amplitude of the IC TQF spectra was greatest at a τ value (~4 ms) that was significantly shorter than that for the EC TQF spectra (τ_{max} ~12 ms). Extrapolation to the situation without SR indicates that this difference would be even greater, since τ_{max} for the shifted EC spectra was ~50% shorter than τ_{max} for the calculated SR-free EC spectra (~12 versus ~24 ms, respectively). These findings indicate that the average molecular order of IC Na is greater than that for EC Na, so that detection of IC Na is favored at short τ. The discrepancy between our results and a previously reported similarity in SQ IC and EC transverse relaxation times in perfused hearts (18) may be due to alteration of Na relaxation by the SR used in the former study [Dy $(PPP)_2^{7-}$]. In addition, the EC transverse relaxation times reported in our study were determined from direct observation of EC TQF spectra, as opposed to an approximation used in the former study in which EC SQ spectral amplitudes were estimated using SR. The IC transverse relaxation times reported in the current study are similar to values previously reported, using SR-aided SQ, DQF, and TQF Na NMR (10, 11, 18, 25).

The poorer fit of the amplitudes of the EC TQF spectra to Eq. [2] (Fig. 2) suggests that a minimum of at least two different sets of transverse relaxation times is required to describe EC Na relaxation. A distribution of transverse relaxation times may be produced by a range in average distances between Tm^{3+} and EC Na. As suggested by Albert and colleagues (26), the EC Na population may be exposed to variable concentrations of SR, resulting from variable densities of cells and negatively charged macromolecules in the EC space. Alternatively, a range of SR-independent relaxation times may exist for the EC space, reflecting some heterogeneity in the motional hindering of EC Na. Consistent with this latter proposal is the observation that a poor fit (data not shown) to Eq. [2] is obtained for the amplitudes of TQF spectra acquired in the absence of SR (i.e., for fitting of the relaxation time curve to data consisting of at least two different sets of T_{2s} and T_{2f}, which are those for IC and EC Na).

The relative efficiency of generation of the IC and EC TQF signals was determined by substituting τ_{max} for IC Na (4 ms) and the transverse relaxation times in Table 2 (obtained in the presence of SR) in Eq. [2]. This calculation produced similar values for the difference of exponentials in Eq. [2] for IC and EC Na (0.77 and 0.71, respectively). In the absence of SR, the ratio of T_{2s} to T_{2f} for EC Na, as well as the magnitude of these relaxation times, would be greater than in the case with SR (because SR attenuates spectra and decreases transverse relaxation times). Therefore, extrapolation to the situation without SR indicates that the value of the difference of exponentials in Eq. [2] for EC Na would be greater than 0.71. The limit of this function and, hence, its contribution to the result of Eq. [2], will approach unity at higher ratios of T_{2s} to T_{2f} [the independence of DQF spectral amplitude to higher ratios of T_{2s} to T_{2f} has been previously reported (27)]. Thus, in the presence of SR, the use of τ_{max} for IC Na and the shorter IC transverse relaxation times do not confer a significant advantage in terms of sensitivity for IC over EC Na, and any advantage is likely nonexistent in the absence of SR. It is clear from this analysis that differences in transverse relaxation, both with and without SR, cannot account for the disproportionately increased contribution of IC Na to the total TQF signal (~23-fold greater than EC Na based on Na content). From these results, we infer that a much greater percentage of IC Na exhibits TQ coherence relative to EC Na (which implies that a much greater percentage of IC versus EC Na is bound or in a viscous state, so that M_o in Eq. 2 is disproportionately greater for IC Na). Although relative transverse relaxation times and optimal creation time values of IC versus EC Na can be exploited to monitor IC Na, the amount of biexponentially relaxing Na within cells appears to contribute most to the sensitivity of MQF techniques for IC Na.

Response of IC and EC Na Spectra to Interventions

The magnitude of the increase of the IC TQF spectral amplitudes during ouabain perfusion (~330% after 25 min of exposure to 0.2 m*M* ouabain; Fig. 3b) is similar to that obtained by other investigators, who used SQ NMR under similar conditions (28). No-flow ischemia also caused an appreciable increase in the IC TQF amplitude (~250% relative to base-line conditions after 60 min; Fig. 5b), which is comparable to values previously reported by some investigators, using SR-aided SQ NMR (29–32). In contrast, other investigators (33) have reported only minor increases in the amplitude of the IC DQF Na spectra under similar conditions (~25% after 60 min of ischemia) and a rate of increase of the IC SQ NMR spectral amplitudes that was much greater than that of the IC DQF spectra. This was attributed to either ischemia-induced alterations in transverse relaxation times (also demonstrated in ref. 25) or to an increase in the visibility of the SQ signal relative to the DQF signal. Possible toxicity from the SR used [Dy $(PPP)_2^{7-}$] may also be responsible for the discrepancy between that study and this one. The hypoxia-aglycemia experiments produced an increase in the IC TQF spectral amplitude that was similar to that obtained with the ischemia and ouabain interventions (~280% increase over 50 min, Fig. 4b). Hypoxia was combined with aglycemia in this study, because this combination has previously been shown to significantly increase IC Na content in rabbit ventricular muscle (34), and prior work in our laboratory and by others (35, 36) has shown that hypoxia alone has only modest effects in increasing IC Na in perfused rat hearts.

Thus, the IC TQF Na spectral amplitude responded to interventions expected to increase IC Na content. Whether changes in MQF spectral amplitude correlate directly with changes in IC Na content remains to be determined, especially given possible changes in transverse relaxation induced by an intervention such as ischemia. However, it should be noted that the amplitude of the DQF spectrum has been shown to be an accurate representation of Na content in phantom models at physiologically relevant Na concentrations (37). In addition, the amplitude of the IC DQF spectrum has been shown to mirror that of the IC SQ spectrum for a change in IC Na content in human erythrocytes (38), which suggests that similar behavior may be exhibited in more complex biological systems. The general correlation between the

magnitude of increase in the IC TQF spectral amplitudes that we report here and prior SQ results also suggests that TQF amplitudes generally correlate with changes in IC Na content.

The behavior of the EC TQF spectral amplitudes varied between different types of interventions. For the constant perfusion interventions (ouabain and hypoxia-aglycemia), the amplitude of the EC TQF spectra remained essentially constant (±20% and ±10% relative to baseline, respectively; Figs. 3b and 4b). Inasmuch as the efficiency of generation of the TQF signal is similar for IC and EC Na, this indicates that the EC Na pool exhibiting TQ coherence must be stable. The EC Na pool contributing to the EC TQF signal is probably maintained during constant tissue perfusion.

During no-flow ischemia, the initial decrease in EC TQF spectra (~33% in the first 20 min of ischemia; Fig. 5b) may be attributed to loss of EC fluid from both the vascular and interstitial spaces (39). Additionally, the decrease in EC TQF spectral amplitude during ischemia could be attributed to uptake of Na by cells, although this was unlikely during the initial period of ischemia before cardiac energy stores were depleted. That this mechanism did not contribute is supported by the fact that the EC TQF spectral amplitude had largely decreased before the IC TQF amplitude had increased appreciably (Fig. 5a). Alternatively, a decrease in the efficiency of EC TQF signal generation due to ischemia-induced changes in transverse relaxation could also account for the initially decreased EC TQF spectral amplitude (an increase in transverse relaxation times for IC Na during ischemia has been previously reported (25)). However, this is unlikely, given that the decrease in spectral amplitude was seen almost immediately after interrupting perfusate flow.

The difference in behavior of the EC Na spectral amplitudes between the constant perfusion interventions and no-flow ischemia suggests that the SR-aided TQF technique used here was sensitive to changes in EC Na content. The stability of the EC spectral amplitudes that we observed during constant perfusion interventions thus serves as indirect proof that EC Na content was stable during these interventions. This can be extrapolated to studies in the absence of SR, provided that in the current study there was no compartment of EC Na for which the spectral amplitude was quenched (and thus, unobservable) and that the behavior of EC Na was similar for populations exhibiting and not exhibiting TQ coherence. The results of the experiments performed without SR during constant perfusion grossly correlate with the results of the experiments with SR. For these interventions, the total TQF spectral amplitude increased ~twofold, less than the increase noted in the IC spectra during the same interventions, which is expected, given the significant but smaller contribution from IC Na to the total TQF spectra.

The lack of increase in the TQF spectral amplitude acquired without SR during no-flow ischemia was expected, given the initial decrease in the EC amplitude. The apparent loss of Na contributing to the EC TQF signal after interruption of perfusion overcomes the ~threefold increase in IC Na that is suggested by the increase in IC TQF spectral amplitudes in the experiments with SR. The discrepancy between the results with TQF NMR reported here and prior results showing increases in total DQF spectral amplitudes during ischemia (11), may be explained by the contribution of a second rank tensor to the EC DQF signal. Destructive interference between signals derived from second and third rank tensors markedly attenuates the EC spectra, so that the relative contribution from IC Na to the total DQF spectral amplitude is greater than that of TQF amplitudes (40).

Assessment of the Potential of MQF ^{23}Na NMR in Monitoring a Change in IC Na in the Absence of SR

This work has demonstrated that IC Na makes a significant contribution to the total TQF spectrum, due to a much greater proportion of IC relative to EC Na exhibiting TQ coherence. The disproportionately increased signal from IC Na allows greater sensitivity of TQF versus SQ NMR to monitor changes in IC Na content. During interventions that used constant perfusion, the EC spectral amplitude was essentially constant, so that the change in the amplitude of the total TQF spectrum (i.e., in the absence of SR) would be expected to be from the change in IC Na content. The magnitude of the increase of the total TQF spectral amplitude in the absence of SR during these interventions is consistent with this premise. This suggests that TQF Na NMR may be able to monitor changes in IC Na in the absence of SR during constant perfusion interventions. In contrast, the fall in the EC TQF spectral amplitude and the lack of increase in the total TQF amplitude in the absence of SR during no-flow ischemia suggest that the use of TQF methods may be more limited in this case. Whether these conclusions also apply to other interventions in other biological systems needs to be determined. MQF techniques in the absence of SR have been used to qualitatively monitor IC Na in a variety of biological systems, including the kidney (13), liver (14), brain (15), heart (11, 16), and salivary gland (17). The results of this study provide a theoretical and experimental foundation for use of this approach. Further experiments, using independent measures of IC Na content, are required to correlate changes in MQF spectral amplitude with changes in IC Na content to test the degree to which this technique provides quantitative measures of IC Na changes. If such validations can be performed, then MQF techniques should prove to be a useful noninvasive, nontoxic method of monitoring changes in IC Na content in myocardium and other tissues.

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