Thulium 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetrakis(methylene phosphonate) as a ^{23}Na Shift Reagent for the *in Vivo* Rat Liver†

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ABSTRACT: The use of thulium 1,4,7,10–tetraazacyclododecane-1,4,7,10-tetrakis(methylene phosphonate ($TmDOTP^{5-}$) as an *in vivo* ^{23}Na NMR shift reagent for rat liver was evaluated by collecting interleaved ^{23}Na and ^{31}P spectra. Infusion of 80 mM $TmDOTP^{5-}$ *without* added Ca^{2+} produced baseline-resolved peaks from intra- and extracellular sodium without producing any changes in phosphate metabolite resonances or intracellular pH. Several key physiological parameters measured in parallel groups of animals confirmed that liver physiology is largely unaffected by this shift reagent. A direct comparison of $TmDOTP^{5-}$ versus $DyTTHA^{3-}$ showed that after infusion of 5–8 times more $DyTTHA^{3-}$, the extracellular sodium peak shifted by the same amount as with $TmDOTP^{5-}$, but the two ^{23}Na resonances were very broad and not resolved. The baseline-resolved peaks with $TmDOTP^{5-}$ allowed us to measure the *in vivo* T_1 and T_2 relaxation characteristics of intra- and extracellular Na^+. The measured T_1, T_{2s}, and T_{2f} values and the relative contributions from the slow and fast T_2 components for intracellular Na^+ in liver did not differ significantly from the values reported for perfused frog heart. The T_1 and T_2 relaxation curves of the extracellular Na^+ resonances fit a monoexponential function. Analysis of the relative contribution of the fast- and slow-relaxing T_2 components from intracellular Na^+ resulted in a calculated visibility factor of 69 ± 4% and the intracellular Na^+ concentration calculated from the NMR peak intensity ratio, the measured visibility factor, and literature values of intra- and extracellular volume was 19 mM. These results indicate that $TmDOTP^{5-}$ promises to be quite useful as an *in vivo* shift reagent for liver and other organs.

A Na^+ gradient is critically important to many cell functions and is sensitive to disease; consequently there is a continuing interest in methods which differentiate sodium in various tissue compartments. Although nuclear magnetic resonance (NMR)[1] is a convenient, relatively sensitive, nondestructive method for detecting sodium in biological tissue, the usual one-pulse experiment suffers from the fact that ^{23}Na resonances from various tissue compartments are isochronous and hence ion concentration gradients and ion fluxes cannot be monitored. At least three NMR methods have been proposed to solve this problem, methods based on relaxation time differences (Hutchison & Shapiro, 1991; Lee et al., 1992), multiple quantum filters (MQFs) (Pekar et al., 1987; Allis et al., 1991; Lyon et al., 1991), and the use of anionic paramagnetic shift reagents (SRs) (Gupta & Gupta, 1982; Chu et al., 1984; Buster et al., 1990; Bansal et al., 1992). Each approach has disadvantages, especially for *in vivo* applications. It is now clear that the methods based on relaxation time differences and MQFs do not accurately filter intra- versus extracellular signals (Hutchison et al., 1990). In addition, the time required for data collection with these techniques limits their utility (Griffey et al., 1990; Allis et al., 1991; Lee et al., 1992). The primary disadvantage of paramagnetic SRs concerns possible acute toxicity (Matwiyoff et al., 1986; Endre et al., 1989; Ramasamy et al., 1990; Boulanger et al., 1992). Since most SRs for biological cations are by necessity anionic and bind competitively with all biological cations, they could unknowingly compromise the physiology of an organ by disrupting normal Ca^{2+}, Mg^{2+}, Na^+, or K^+ ion gradients. Despite this disadvantage, SRs do allow simultaneous measurement of ^{23}Na signals from multiple tissue compartments so that relative changes in Na^+ ion concentrations can be detected in various compartments with excellent temporal resolution.

A number of different SRs have been successfully used to monitor intracellular sodium in isolated cells and perfused tissue (Gupta & Gupta, 1982; Chu et al., 1984; Buster et al, 1990) but only dysprosium(III) triethylenetetraaminehexaacetate ($DyTTHA^{3-}$) (Albert et al., 1990; Naritomi et al., 1987; Balschi et al., 1990; Blum et al, 1991) and thulium(III) 1,4,7,-10-tetraazacyclododecane-1,4,7,10-tetrakis(methylene phosphonate) ($TmDOTP^{5-}$) (Kohler et al., 1992; Bansal et al., 1992) have been used *in vivo*. Previously, we have used $TmDOTP^{5-}$-aided ^{23}Na chemical shift imaging in combination with ^{23}Na and ^1H imaging to monitor Na^+ in successive 1-mm slices in the rat brain *in vivo* and have demonstrated that, like various relaxation agents used in MRI, the SR does not cross the blood brain barrier (Bansal et al., 1992). In the present

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[1] Abbreviations: NMR, nuclear magnetic resonance; MQF, multiple quantum filter; SR, shift reagent; TTHA, triethylenetetraaminehexaacetate; DOTP, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylene phosphonate); MRI, magnetic resonance imaging; FID, free induction decay; T_1, spin–lattice relaxation time; T_2, spin–spin relaxation time; MAP, mean arterial pressure; PPP, tripolyphosphate; E_m, transmembrane potential; T_{2s}, slow spin–spin relaxation time; T_{2f}, fast spin–spin relaxation time; DTPA, diethylenetriamine pentaacetate.

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study, we evaluate the use of TmDOTP^{5-} in discriminating between different sodium compartments in the liver in live animals and report, for the first time, the *in vivo* relaxation characteristics of intra- and extracellular sodium.

MATERIALS AND METHODS

Shift Reagents. Stock solutions of TmDOTP^{5-} (80 mM) were prepared as described in Bansal et al. (1992). DyTTHA^{3-} was prepared by mixing $DyCl_3$ with a slight molar excess of TTHA (Sigma Chemical Co., St. Louis, MO) in water and titrating with NaOH to a pH of 7.4. The final concentration of DyTTHA^{3-} was 400 mM.

Animal Preparation. Male Sprague-Dawley rats weighting 350–450 g were initially anesthetized by intramuscular injection of a 0.5-mL mixture of ketamine (13 mg/mL) and xylazine (87 mg/mL). Both jugular veins and a carotid artery were cannulated through a midline neck incision. One jugular vein was used to maintain the anesthesia (2 mg/mL ketamine and 0.25 mg/mL xylazine in 5% guaifenesin) at a rate of 2–3 mL/hour, and the other was used to infuse SR. The blood pressure and heart rate were continuously monitored from the carotid artery during the NMR experiments using a Gould transducer and Coulbourn polygraph. A tracheotomy was performed and the rat was connected to a respirator and maintained at a respiration rate of 90 breaths/min and a tidal volume of 3 cm^3. The rats were also nephrectomized to eliminate clearance of the shift reagent, except in those animals where SR clearance was measured. Livers were exposed through a subcostal incision and a surface coil was positioned directly on the liver with the animal in a supine position. The animals were maintained at 37 °C using a water-recirculating heating pad.

SR Infusion. Stock solutions of 80 mM TmDOTP^{5-} or 400 mM DyTTHA^{3-} were initially infused at a rate of 2 mL/h for 6 min. The rate was slowly increased to 8 mL/h over 6 min and then maintained at this level for 20–30 min. After a chemical shift difference of 5–7 ppm between the intra- and extracellular sodium resonances was achieved the infusion rate was reduced to 2–3 mL/h. No Ca^{2+} was added during infusion of either SR.

NMR Data Collection. All *in vivo* NMR experiments were performed on a 4.7-T 40-cm GE CSI Omega spectrometer (GE NMR Instruments, Fremont, CA). Shimming was performed on the sodium signal. A sodium line width of 35–40 Hz was typical. Both ^{23}Na and ^{31}P spectra were collected in an interleaved fashion during TmDOTP^{5-} infusion. A 2.3-cm diameter surface coil was dual-tuned to 53 MHz for ^{23}Na and 81 MHz for ^{31}P, using a circuit design as described in Schall et al. (1986). The coil performance was optimized such that there was less sensitivity loss at the ^{31}P frequency as compared to at the ^{23}Na frequency. For ^{23}Na, a 50-μs excitation pulse was followed by a 10-μs dead time and 1024 real data points were collected over a sweep width of 3000 Hz with the preamplifier filter turned off. For ^{31}P, a 30-μs pulse was followed by a 100-μs dead time and 2048 real data points were collected over a sweep width of 5000 Hz with the preamplifier filter on. Switching of these parameters and the spectrometer frequency was executed by a script in an automated fashion. To take advantage of the short spin–lattice relaxation time of sodium, two ^{23}Na acquisitions were collected between each ^{31}P acquisition. Cyclops phase cycling was used for both the nuclei. The minimum repetition time for ^{23}Na was 0.34 s and for ^{31}P was 3.66 s. The data were initially collected as a sum of 64 acquisitions for ^{23}Na and 32 acquisitions for ^{31}P over 1.95-min intervals. For the data presented in Figure 2, four consecutive ^{31}P free induction decays (FIDs) were summed, resulting in 128 acquisitions per spectrum collected over a 7.8-min period. The FIDs were Fourier-transformed after baseline correction and multiplication by a single-exponential corresponding to 10-Hz line broadening for ^{23}Na and a 20-Hz line broadening for ^{31}P.

^{23}Na spin–lattice (T_1) and spin–spin (T_2) relaxation times were measured before and after TmDOTP^{5-} infusion using a 2-cm surface coil single tuned to 53 MHz. The instrument dead time was set to 10 μs for all relaxation experiments. A pulse–burst saturation recovery experiment was performed using 50 saturation pulses (23 μs) followed by an incremental delay (15 values ranging from 1 to 256 ms) and a 23-μs pulse and acquisition with cyclops phase cycling for measurement of T_1. A Hahn spin–echo experiment with exorcycle phase cycling was used for measurement of T_2. A 23-μs exciation pulse and a 46-μs refocusing pulse were used, and the spin echo time was varied from 0.05 to 40 ms in 25 steps. The insrument dead time of 10 μs was included as a part of the echo time. One thousand twenty-four real data points were collected over a sweep width of 3000 Hz and either 128 or 256 transients were acquired at each delay for both T_1 and T_2 experiments. Each T_1 was measured five times on three rats (two animals were studied twice) and each T_2 was measured nine times on five rats (four animals were studied twice). The relaxation times were computed by fitting the peak integral of the resonances to both mono- and biexponential functions. Measurement of T_2 with a surface coil was validated by comparing the T_2 values of a normal saline solution determined using a volume coil versus a surface coil. The T_2 values obtained by the two coils were identical. The relaxation times of extracellular Na^+ in the absence of TmDOTP^{5-} were calculated by subtracting the raw relaxation curves of intracellular Na^+ from the corresponding raw relaxation curves of total Na^+ without SR. The subtracted curves were then fit to mono- and biexponential functions. These calculations assume that the presence of TmDOTP^{5-} in extracellular space does not change the relaxation time of intracellular Na^+. The fact that we observe no change in the intracellular ^{23}Na line width during infusion of increasing quantities of TmDOTP^{5-} (described below) suggests this assumption should be valid. Also, Burstein and Fossel (1987) have shown that doubling the concentration of dysprosium(III) bistripolyphosphate [$Dy(PPP)_2^{7-}$] did not change the relaxation times of intracellular Na^+ in perfused frog heart.

RESULTS

Physiological Measurements. Several key physiological measurements were obtained on a group of animals ($n = 7$) during infusion of TmDOTP^{5-} using the same protocol as used during all subsequent NMR measurements. No significant changes in heart rate (240 ± 20) or developed pressure (40 ± 5 mmHg) were observed, but the mean arterial pressure (MAP) tended to decrease from about 90 to 70 mmHg during infusion of the SR (see Figure 1). The decrease in MAP appeared to parallel a relatively small change in free Ca^{2+} levels in blood serum as detected by a calcium-specific electrode. It should be noted that excess Ca^{2+} was *not* coadded with the SR in these experiments, unlike in isolated perfused heart experiments where excess Ca^{2+} must be coadded with TmDOTP^{5-} to maintain an extracellular free [Ca^{2+}] of about 1 mM (Buster et al., 1990). We have previously shown that Ca^{2+} is rapidly released from body stores during infusion of TmDOTP^{5-} into live rats (Bansal et al., 1992).

The resting transmembrane potential ($-E_m$) was measured in *in vivo* exposed livers using a modified Ling–Gerard

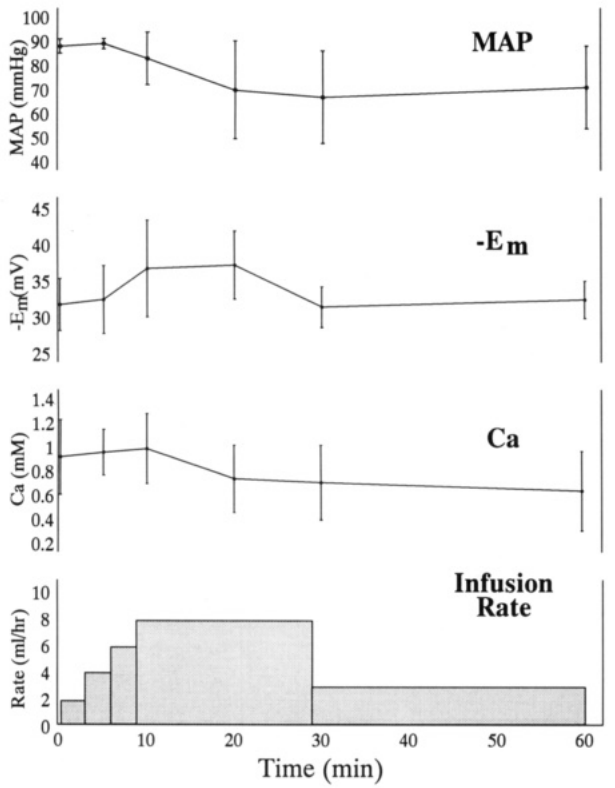

FIGURE 1: Plots of mean arterial pressure (MAP), resting liver transmembrane potential ($-E_m$) and free Ca^{2+} levels in serum during infusion of 80 mM $TmDOTP^{5-}$ at the rates indicated in the lower panel. The error bars represent ±1 standard deviation (n = 7).

ultramicroelectrode (Holliday et al., 1981), and serum sodium, potassium, and hemoglobin concentrations as well as arterial pH, pCO_2, and pO_2 were monitored throughout the infusion protocol using a standard clinical blood gas analyzer. The resting transmembrane potential tended to increase during the highest infusion rates (Figure 1) and then return to basal levels during lower levels of steady-state infusion of the SR. Interestingly, serum sodium increased by only 10 mequiv/L during the entire infusion protocol (from 149 ± 3 to 158 ± 5 mequiv/L) even though the total amount of sodium infused with the SR was substantial. Serum potassium (3.5 ± 0.2 mequiv/L), hemoglobin (13 ± 1 g/dL), pH (7.36 ± 0.02), pCO_2 (45 ± 2 mmHg) and pO_2 (120 ± 8 mmHg) remained unchanged throughout the protocol. These results suggest that basic liver physiology is largely unaffected by $TmDOTP^{5-}$ at the doses required for baseline resolution of the intra- and extracellular ^{23}Na resonances (see below).

^{23}Na Shift Experiments. Interleaved ^{23}Na and ^{31}P spectra collected during $TmDOTP^{5-}$ infusion are shown in Figure 2. Note that unlike in the ^{23}Na brain study (Bansal et al., 1992), where two shifted resonances appeared early during the infusion (assigned to vascular and interstitial Na^+), the paramagnetically shifted ^{23}Na resonance in the *in vivo* liver remains a single symmetrical resonance throughout the infusion period. The intra- and extracellular ^{23}Na resonances were clearly resolved after 0.55 mmol kg^{-1} (body weight) of $TmDOTP^{5-}$ had been introduced. After a 0.65 mmol kg^{-1} dose of SR, the extracellular Na^+ was paramagnetically shifted by approximately 5 ppm. Once discernible from the extracellular Na^+ peak, the intracellular Na^+ peak intensity did not change with further infusion of SR, suggesting that $TmDOTP^{5-}$ does not alter the intracellular Na^+ concentration in the liver at these dosages.

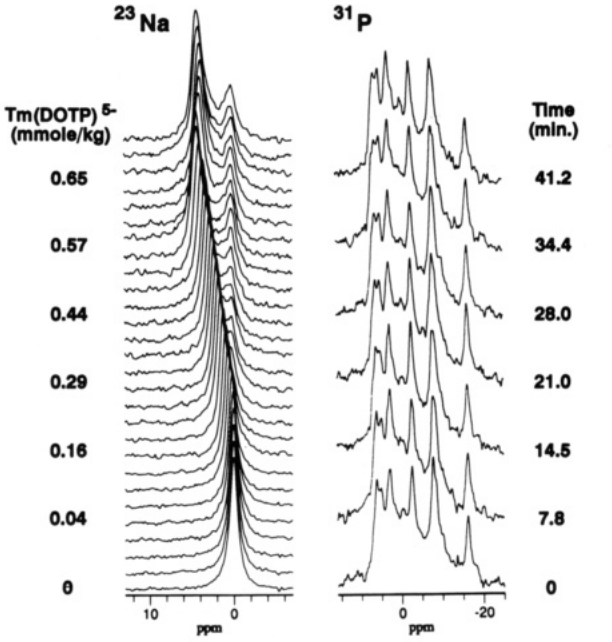

FIGURE 2: Stacked plots of interleaved ^{23}Na (left) and ^{31}P (right) spectra from rat liver in vivo collected during $TmDOTP^{5-}$ infusion. The time indicated at the right of each ^{31}P spectrum is at the beginning of data acquisition. The infusion doses at the corresponding time intervals are shown at the left of ^{23}Na spectra.

The ^{31}P spectra collected before and during the $TmDOTP^{5-}$ infusion showed three ATP resonances, a single inorganic phosphate resonance, and one sugar phosphate resonance. The intensity of the phosphocreatine resonance was quite small, indicating that the spectra were mainly from the liver and did not have any significant contributions from the surrounding tissue. The ^{31}P spectra also showed an underlying broad signal. This broad signal has been reported by other investigators (Malloy et al., 1981; Bates et al., 1989) and has been assigned to phosphodiesters in the phospholipid bilayer (Murphy et al., 1989). The ^{31}P resonance areas and chemical shifts were unaffected by the SR, indicating that $TmDOTP^{5-}$ does not alter the cellular energy state of the liver. The intracellular pH calculated from the shift of inorganic phosphate peak was also unaffected by the SR. The ^{23}Na and ^{31}P spectra shown in Figure 2 were collected using the double-tuned 2.3-cm surface coil described in Materials and Methods. ^{23}Na spectra were also collected using a 1-cm surface coil and these showed a very similar ratio of intra- an extracellular Na^+ (data not shown). Once again, this suggests that the 2.3-cm coil used in this work is sampling only liver tissue and not some average of liver, muscle, and/or intestine.

A direct comparison of $TmDOTP^{5-}$ versus $DyTTHA^{3-}$ as a ^{23}Na shift reagent in the *in vivo* liver is shown in Figure 3. Although, the animals remained hemodynamically more stable during infusion of $DyTTHA^{3-}$, this SR induces a much lower ^{23}Na shift per unit concentration and more severe line broadening than does $TmDOTP^{5-}$. The dose of $DyTTHA^{3-}$ required to induce a shift of 5 ppm was 3.74 mmol kg^{-1} (body weight) as compared to 0.65 mmol kg^{-1} $TmDOTP^{5-}$. Also, the peak width at half height for the intra- and extracellular resonances when separated by 5 ppm were 195 and 300 Hz, respectively, for $DyTTHA^{3-}$ but only 70 and 80 Hz for $TmDOTP^{5-}$. The greater line widths produced by $DyTTHA^{3-}$ result from a combination of larger bulk magnetic susceptibility of Dy(III) versus Tm(III) and the higher concentration of $DyTTHA^{3-}$ required to separate the intra- and extracellular resonances. The fact that the line width of the intracellular resonance is broadened by nearly a factor of 3 for $DyTTHA^{3-}$

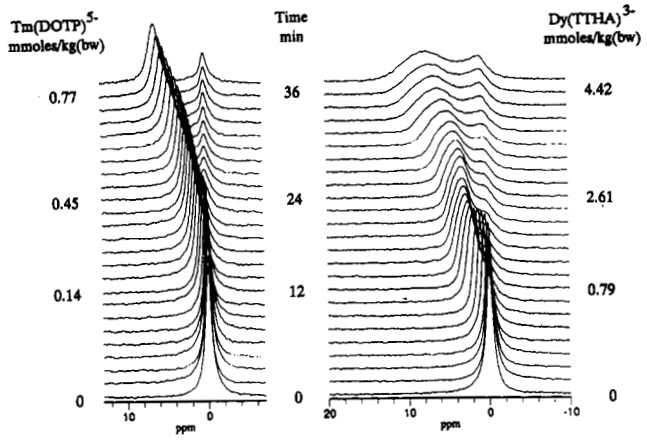

FIGURE 3: Comparison of ^{23}Na spectra from rat liver during TmDOTP^{5-} (left) and DyTTHA^{3-} (right) infusion. The time indicated in the middle of the stacked plots is at the beginning of data acquisition. The infusion doses at the corresponding time intervals are shown at the left for TmDOTP^{5-} and at the right for DyTTHA^{3-}.

versus TmDOTP^{5-} indicates that bulk susceptibility broadening is particularly severe with the Dy(III) reagent. Infusion of higher doses of DyTTHA^{3-} did not improve the resolution of intra- and extracellular resonances because higher doses increased the line widths even further.

The washout rate of TmDOTP^{5-} was measured by monitoring the frequency of the shifted ^{23}Na peak after stopping the infusion. The chemical shift versus time data (not shown) fit to an exponential decay function yielded a $t_{1/2}$ of 9 min (n = 2). This half-life is shorter than the $t_{1/2}$ of 37 min for washout of TmDOTP^{5-} from rat brain (Albert et al., 1990) and a $t_{1/2}$ of 44 min for washout of DyTTHA^{3-} from rat muscle (Balschi et al., 1990). A recent CSI study (Bansal et al., 1992) indicated that most of the shifted ^{23}Na signal detected by a surface coil in a rat brain experiment actually arises from Na$^+$ in muscle surrounding the skull and hence the agreement between the muscle and brain studies would be expected. The significantly shorter $t_{1/2}$ we measured for washout of TmDOTP^{5-} from the liver interstitium likely reflects greater blood flow in liver relative to resting muscle.

T_1 and T_2 Relaxation Measurements. All relaxation measurements were obtained on animals where the intra- and extracellular ^{23}Na resonances were baseline-resolved (such as that shown in Figure 3). The results of those measurements are summarized in Table I. The relaxation times of extracellular Na$^+$ in the absence of TmDOTP^{5-} were calculated by the subtraction method described in Materials and Methods. Theory predicts that both T_1 and T_2 for a spin $^3/_2$ nuclei such as ^{23}Na are biexponential in tissues where the correlation time is not short compared to the Larmor period; the theoretical ratio of fast to slow relaxing components is 20:80 for T_1 relaxation and 60:40 for T_2 relaxation (Hubbard, 1970). However, all of our experimental T_1 relaxation curves and extracellular T_2 relaxation curves (without SR) fit a single-exponential function rather well. The two T_1 relaxation components may not be separable because their values may differ by less than an order of magnitude or because the fast component accounts for only 20% of the total signal intensity and is therefore more difficult to detect experimentally. Figure 4 compares the semilogarithmic plots of T_2 decay for intracellular and purely extracellular Na$^+$ (calculated by subtraction). The intracellular plot shows a two-component curve, while the purely extracellular Na$^+$ plot shows a straight line indicating a single-component decay. The observed monoexponential T_2 decay for extracellular sodium in rat liver *in vivo* agrees with the observed single-exponential T_2 decay in human serum (Shinar & Navon, 1986) and contrasts with the reported biexponential T_2 decay of interstitial sodium in perfused frog and rat hearts (Foy & Burstein, 1990). The shifted extracellular liver ^{23}Na resonance clearly has significant contributions from both serum sodium and interstitial sodium. Our observation of a single, symmetrical extracellular sodium resonance both during infusion of TmDOTP^{5-} and during washout of the agent indicates that Na$^+$ ion exchange between the vascular and interstitial space in liver is quite rapid. Thus, our measured T_1 and T_2 values for this resonance likely represent an average of the relaxation rates of sodium in the two compartments. Our failure to detect two T_2 components in the extracellular ^{23}Na resonance does not exclude the possibility that both fast and slow components are indeed present. It is possible that the time constants for the two components do not differ significantly [Shinar and Navon (1986) have shown that a single exponential would be observed if $T_{2s}/T_{2f} \leq 2$] or that the short T_2 component is not detected because of particular exchange conditions between the vascular and interstitial spaces. The relative contributions of the two T_2 relaxation components of the combined intra- and extracellular resonance was 16:84 (fast:slow) before SR infusion and 42:58 for the resolved intracellular resonance after SR infusion. The apparent decrease in fast-component contribution for the combined resonance before SR infusion as compared to the intracellular resonance is consistent with monoexponential T_2 decay (slow component only) of extracellular Na$^+$ in the absence of shift reagent.

The values of T_1, T_{2f}, and T_{2s} found here for intracellular Na$^+$ in liver are nearly identical to the respective relaxation times reported for intracellular Na$^+$ in perfused frog hearts (Burnstein & Fossel, 1987; Foy & Burnstein, 1990). Interestingly, the relative contributions from the fast and slow T_2 components for intracellular Na$^+$ were also nearly the same in the two tissues (42:58 in liver versus 48:52 in frog hearts) and neither agrees with the theoretically expected 60:40 ratio for a single pool of intracellular Na$^+$ experiencing biexponential relaxation due to quadrupolar effects. This discrepancy [discussed in detail in Burnstein and Fossel (1987)] could arise from several slightly different pools of sodium with a distribution of fast and slow relaxation times each contributing to the intracellular resonance, where some of the fast-relaxing components decay so rapidly that their detection is partly or completely missed even with echo times as short as 50 μs. If we assume that the slow-relaxing component is 100% visible but some of the rapidly decaying components are not visible (for either chemical or instrumental reasons), then the experimental fast:slow ratio of 42:58 (or 29:40 after normalizing the slow component to 40%) implies that the visibility of *total* intracellular sodium is 29 + 40 = 69 ± 4% (n = 9). Blum et al. (1991) have also reported that intracellular Na$^+$ in liver is 53 ± 21% visible. One should note however that there may be considerable error in our estimated 69% visibility because different pools of sodium ions may be exchanging with each other with different time constants and this could significantly affect the T_{2f} relaxation contribution. However, the observed ratio of 29:40 for the fast:slow components, clearly suggests that there is substantial amount of intracellular sodium in liver that is invisible with a 50-μs spin–echo time or in a one-pulse experiment with a dead time of 10 μs. This is in contrast with the near 100% visibility found for intracellular sodium in Na$^+$-loaded yeast cells (Rooney & Springer, 1991), using spectrometer dead times of less than ca. 25 μs.

Table I: Experimental ^{23}Na Relaxation Times and Percentages of Fast and Slow T_2 Components in Rat Liver

shift reagent		T_1, ms	% $T_{2(slow)}$	$T_{2(slow)}$, ms	% $T_{2(fast)}$	$T_{2(fast)}$, ms
absent	combined intra- and extracellular	33.8 ± 0.3	84 ± 3	17.3 ± 0.8	16 ± 3	1.8 ± 0.3
present	shifted peak	23.9 ± 1.0	100	10.0 ± 2.8		
present	intracellular	21.1 ± 0.6	58 ± 3	13.0 ± 0.9	42 ± 3	1.3 ± 0.1
absent	extracellular (calcd)	41.0 ± 1.0	100	18.0 ± 0.8		

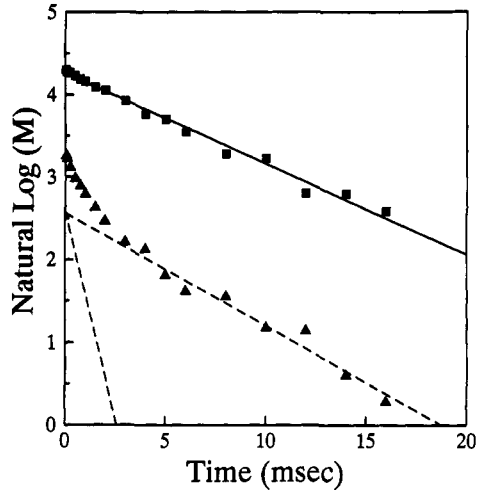

FIGURE 4: Semilogarithmic plot of T_2 relaxation curve for intracellular (▲) and purely extracellular (■) sodium. The purely extracellular sodium curve was calculated by the subtraction method described in the text.

DISCUSSION

This study demonstrates that $TmDOTP^{5-}$ can produce baseline-resolved intra- and extracellular ^{23}Na resonances in the *in vivo* rat liver at relatively low doses of SR. The physiological measurements reported here indicate that this amount of SR is unexpectedly quite nonperturbing to the animal, even though the osmotic load is high. This dose is about 4–6 times greater than a typical dose of MRI contrast agent (0.1–0.2 mmol kg^{-1}), such as gadolinium(III) diethylenetriaminepentaacetate ($GdDTPA^{2-}$). We are aware of only one other ^{23}Na NMR study of *in vivo* liver using a SR. Blum et al. (1991) infused 6 mmol kg^{-1} h^{-1} $DyTTHA^{3-}$ for 60–90 min to discriminate between intra- and extracellular sodium. This SR dose is 8–12 times larger than the amount of $TmDOTP^{5-}$ used here. After $DyTTHA^{3-}$ infusion, their extracellular Na^+ signal shifted downfield by about the same amount as in our study, but the sodium resonances were very broad and were *not* resolved. This demonstrates that of the SRs reported so far, only $TmDOTP^{5-}$ can produce a baseline separation of intra- and extracellular ^{23}Na resonances in liver and this is extremely important for valid measurement of tissue volumes, relaxation rates, and ^{23}Na visibility during physiological interventions. $TmDOTP^{5-}$ also allows interleaved acquisition of ^{23}Na and ^{31}P NMR spectra without producing any significant line broadening in the ^{31}P spectrum.

Integration of the two resolved ^{23}Na resonances gave a relative area ratio of 72:28 (extracellular:intracellular). A wide range of values have been reported for intra- and extracellular volume ratios and Na^+ concentrations in liver (Blum et al., 1991). If one assumes that the V_{int}/V_{ext} volume ratio of 4.6 reported by Willams et al. (1971) is reasonably representative of liver, then our observed peak area ratio of 72:28 gives the Na_{int}/Na_{ext} concentration ratio of 0.12 ± 0.04 after correction for 69% visibility of the intracellular signal. Our measured extracellular Na^+ was 158 mM and therefore the intracellular concentration of Na^+ in liver as detected by NMR is ~19 ± 5 mM. This value agrees with previously reported values measured using a variety of techniques (Williams et al., 1971; Lambotte, 1977; Holliday et al., 1981; Blum et al., 1991).

As reported in Table I, the T_1s for intra- and extracellular sodium calculated by subtraction are 21.1 ± 0.6 an 41 ± 1 ms, respectively. In contrast to the large differences in T_1 relaxation times of ^{31}P metabolites in liver versus heart (Evelhoch et al., 1985), the measured ^{23}Na T_1 values in liver do not differ significantly from the T_1 values in perfused heart (Foy & Burstein, 1990). It has been suggested that hepatic ^{31}P T_1s are much shorter due a higher concentration of paramagnetic ions in liver (Evelhoch et al., 1985), so if this is true, the results presented here indicate that the ^{23}Na T_1s in liver are unaffected by these same paramagnetic ions.

Our data suggest that $TmDOTP^{5-}$ can safely be used in intact animal studies without coaddition of Ca^{2+}. $TmDOTP^{5-}$ induces a larger ^{23}Na chemical shift per unit concentration due to a combination of larger negative charge as compared to $DyTTHA^{3-}$ and a more favorable Na^+ binding site geometry. Furthermore, both the shifted and unshifted signals are sharp and well-resolved with $TmDOTP^{5-}$ because of smaller magnetic susceptibility of Tm(III) as compared to Dy(III). We conclude that $TmDOTP^{5-}$ promises to be quite useful for *in vivo* ^{23}Na NMR studies of liver and perhaps other organs as well.

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