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Intra- and Intermolecular ^1H–^1H Nuclear Overhauser Effect Studies on the Interactions of Chlorpromazine with Lecithin Vesicles

Yoshihiro Kuroda*[1a] and Keisuke Kitamura[1b]

Contribution from the Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan, and Kyoto College of Pharmacy, Misasaginakauchi-cho, Yamashina-ku, Kyoto 607, Japan. *Received June 9, 1983*

Abstract: The interactions of chlorpromazine with sonicated lecithin vesicles have been studied by ^1H–^1H nuclear Overhauser effect (NOE) measurements at 200 MHz. All of the steady-state NOE experiments, in which irradiations were made selectively at the resonances due to the phenothiazine ring and methyl groups of chlorpromazine as well as those due to choline methyls, the glycerol moiety, acyl methylenes, olefinic methines, and terminal methyl groups of lecithin, caused negative intra- and intermolecular NOEs for the remaining unirradiated resonances. These observations indicate unambiguously that even at a resonance frequency of 200 MHz and even in sonicated lecithin vesicles the spin diffusion mechanism is operating and that chlorpromazine, incorporated into the bilayer of lecithin vesicles, moves with the same time scale as that of the vesicles. Although this spin diffusion mechanism is not the dominant relaxation mechanism of the protons in the sonicated lecithin, steady-state NOEs are governed by this phenomenon and consequently result in less specific NOE. We demonstrated that the relative rates of growth of the NOEs obtained from time-dependent NOE experiments represent spatial arrangements of the protons in the lecithin molecules which interacted with chlorpromazine. The results confirmed that the phenothiazine ring of chlorpromazine is located in the immediate neighborhood of the chain methylenes α to the carbonyl carbons and that the positively charged *N*-dimethyl group is interacting with the phosphate in the polar headgroup of lecithin.

An understanding of drug interactions with membranes at the molecular level is of great importance in the field of pharmacology. Nuclear magnetic resonance (NMR) spectroscopy is a well-suited technique to examining this problem and has been extensively applied to various combinations of drug-membrane systems.[2] The NMR parameters (or techniques) so far employed to study the interactions were chemical shifts, line widths, spin–lattice (T_1), and spin–spin (T_2) relaxation times. The effects of paramagnetic ions on these parameters have also been examined.[2i-k] Addition of a drug to the membrane solutions is expected to cause changes in these parameters of the drug itself and/or the membranes, therefore the interactions can be monitored.

Measurement of the ^1H-^1H nulcear Overhauser effect (NOE) has been shown to be a good method for elucidating the structures of small[3] and large molecules[4] and also the interactions between them.[5] In the present study, we applied this technique to investigate drug–membrane interactions. The drug–membrane systems chosen were chlorpromazine– and nicotinamide–egg yolk lecithin vesicles. Chlorpromazine (I), a tranquillizer, was shown to interact with lecithin vesicles (single bilayers) and liposomes (multibilayers) by electron spin[6] and nuclear magnetic rsonance[2h-k] and calorimetric studies;[2h] the binding site of this drug appears to be the polar region of phospholipids. Nicotinamide (II) was chosen as a reference having no interactions with the lecithin vesicles. Sonicated lecithin vesicles are well suited for applying the NOE technqiue because their ^1H NMR spectra show such well-resolved peaks[7] that we can selectively saturate each resonance

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and can monitor changes in peak intensities in the rest of the spectrum.

Before applying the NOE technique to the above drug–membrane systems, however, one very essential question arises; that is, whether the NOEs due to intermolecular (between the drug and lecithin) and intramolecular (within the drug or within the lecithin) ^1H–^1H dipolar interactions can be observed as positive or negative enhancements at a spectrometer resonance frequency of 200 MHz. It is well recognized that even in the sonicated lecithin vesicles motion of the phospholipid molecules is highly anisotropic[8] and that the magnitudes of NOEs depend on the correlation times of interacting spins.[9] The correlation times in the sonicated vesicles can be divided into two groups; one is relatively short (10^{-10}–10^{-11} s) and the other is long ($\geq 10^{-8}$–10^{-9} s).[8a] The positive NOEs are responsible for the former fast motion and the negative ones for the latter slow motion.[9] Thus, the observable NOEs may be a result of complicated competing contributions from positive and negative enhancements. The present work was undertaken to see whether the observed NOEs simply reflect the distance-dependent dipole–dipole interactions between saturated and observed spins. We will present experimental evidence that steady-state NOEs do not give correct structural information on account of nuclear spin diffusion[10] and that the initial rates of buildup of the NOEs should be measured in order to obtain information on the interproton distances in sonicated lecithin vesicles.

Experimental

Materials. Samples of egg yolk lecithin and chlorpromazine hydrochloride were taken from the same batches of preparations as reported previously.[2i-k] Nicotinamide was obtained from a commercial source. Sonicated vesicle solutions (3%, w/v) were prepared as described previously.[2j]

Measurements. The ^1H NMR experiments were carried out on a JEOL FX-200 spectrometer operating at 200 MHz. Ambient probe temperatures were 23.3–23.8 °C. All the spectra were taken under conditions of 36° pulse flip angle and 8K of memory for 2000-Hz spectral width. Steady-state NOE difference spectra were obtained by subtracting alternatively acquired eight on-resonance free induction decays (FIDs) from off-resonance irradiated FIDs. The preirradiation and pulse delay times for each accumulation were 5 and 2 s, respectively. Truncated driven NOE difference spectra[11] were obtained by varying the preirradiation times from 50 ms to 5 s. Transient NOE difference spectra were obtained as described by Wüthrich et al.[11b,12] The strength of the irradiation radiofrequency field (H_2) was estimated by measuring the duration of the irradiation pulse required to produce 180° inversion of magnetization in the sample solution and is expressed in units of Hz ($\gamma H_2/2\pi$). Spin–lattice relaxation times (T_1) were obtained by the usual $(180°$–t–$90°$–$T)_n$ pulse sequence. The delay time T was 4 s. For a resonance having a T_1 value longer than 1 s, however, the T_1 value was roughly estimated from the time t_0 for zero passage of recovering magnetization using $T_1 = t_0/\ln 2$. In all the spectra, chemical shifts are reported relative to the peak of internal methylenes of lecithin.

Results

Steady-State Nuclear Overhauser Effects. Figure 1a–l shows the steady-state NOE difference spectra of chlorpromazine–lecithin vesicles in D_2O and normal 200-MHz ^1H NMR spectra of (m) lecithin vesicles containing chlorpromazine, (n) lecithin vesicles containing no drug, and (o) chlorpromazine hydrochloride in D_2O. In the spectrum 1m, we labeled prominent peaks A–L and listed

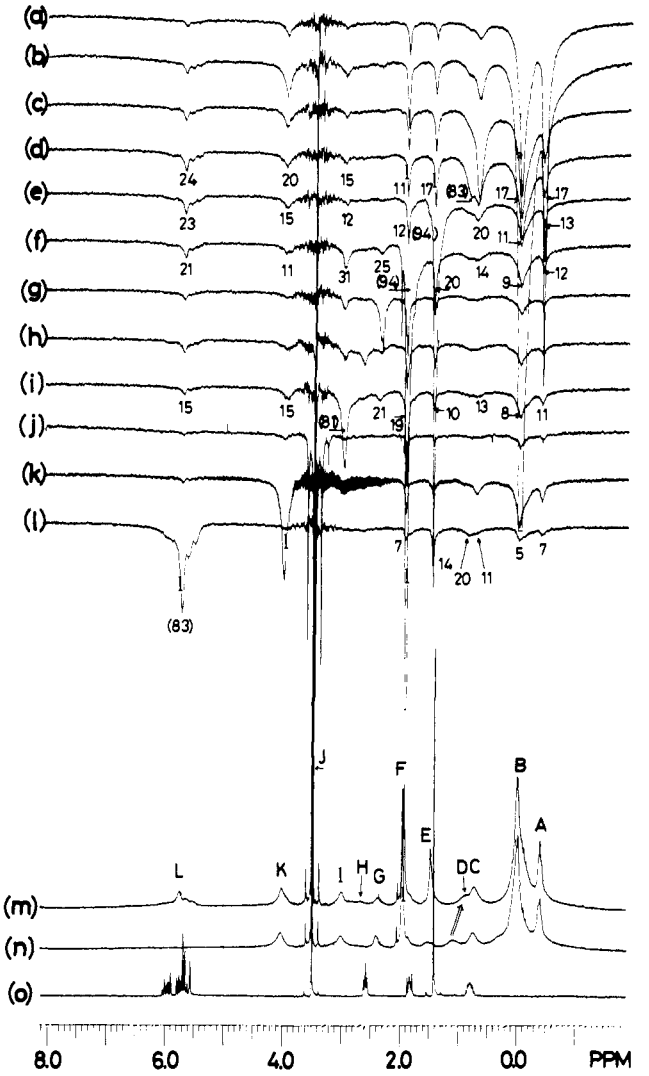

Figure 1. (a–l) Steady-state NOE difference spectra of chlorpromazine (14 mmol dm^{-3})–lecithin vesicles (3%, w/v) in D_2O; (m) ^1H NMR spectrum of the chlorpromazine–lecithin vesicles solution; (n) ^1H NMR spectrum of 3% lecithin vesicles in D_2O; (o) ^1H NMR spectrum of 14 mmol dm^{-3} chlorpromazine hydrochloride in D_2O. In a–l, each spectrum was obtained by irradiating the corresponding peak labeled A–L in m. In d–f, i, and l, the percentage of NOE is shown for each peak; the values in parentheses indicate the extent of saturation.

Table I. Chemical Shifts, Assignments, and T_1 Values of Peaks A–L of Chlorpromazine (14 mmol dm^{-3})–Lecithin Vesicle (3%, w/v) Solution

peak	chemical shifts[a]	assignment	T_1[b]
A	−0.39	CH_3	0.579 (0.585)
B	0.00	$(CH_2)_n$	0.423 (0.416)
C	0.74	$CH_2C{=}C$, $-CH_2-$(CP)[c]	0.383 (0.359)
D	0.88	CH_2CO, $-CH_2-$(CP)[c]	0.320 (0.292)
E	1.49	$NH(CH_3)_2^+$(CP),[c] $=C-CH_2-C=$	0.300 (0.268)
F	1.95	$N(CH_3)_3^+$, CH_2-NH^+(CP)[c]	0.340 (0.333)
G	2.40	CH_2N^+	0.350 (0.378)
H	2.70	$N-CH_2$(CP)[c]	n.d.[e]
I	3.01	CH_2OCO, CH_2OP	0.371 (0.328)
J	3.51	HDO(solvent)	~14
K	4.03	$CH{=}CH$, CHOCO	0.534 (0.536)
L	5.76	Phe(CP)[c,d]	0.490

[a] In ppm, ±0.01 ppm. [b] In s, ±0.01 s. T_1 values measured in the presence of a saturating radio frequency field applied to peak L are in parentheses. [c] (CP): chlorpromazine. [d] Phe: phenothiazine ring protons. [e] Not determined.

their assignments in Table I together with their T_1 values. The resonance assignments of lecithin protons mainly followed those

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of Hauser et al.,[13] some of which were confirmed by the present study. The assignments of chlorpromazine were obvious from the expected chemical shifts. It should be noted that the ^1H NMR spectrum of chlorpromazine hydrochloride in D_2O (spectrum 1o) shows fine structures due to spin–spin couplings. This spectral feature indicates that no micelle formation occurred in the presently employed concentration; the micelle formation was reported previously for phenothiazine derivatives including chlorpromazine and is known to cause line broadening in their ^1H NMR spectra to such an extent that no fine structures can be detected.[14] Thus, the broadening of peaks of chlorpromazine in spectrum 1m can be ascribed to the interactions with lecithin vesicles and not to the micelle formation. Another obvious feature of the interactions is the upfield shift of peak D which arises from α-methylenes in the fatty acid chains of lecithin (indicated as an open arrow from spectra 1n to 1m). This upfield shift has been well discussed by Kitamura et al.[2k] as an indication of the interactions between the phenothiazine ring of chlorpromazine and the α-methylenes of lecithin. The NOE difference spectra (a–l) were obtained under identical conditions of radio frequency power ($\gamma H_2/2\pi \sim 4.2$ Hz) and preirradiation time (5 s) by saturating peaks A–L. A downward peak means a negative NOE, and the precentages are indicated for some of the typical spectra (d–f, i, and l). The percentage was evaluated from the peak heights of on-resonance and off-resonance irradiated ^1H NMR spectra obtained from separate experiments with the same radio frequency power and preirradiation time as those used in the NOE difference experiments. Radio frequency irradiation power was set as low as possible to obtain selectively irradiated NOEs and to afford saturation factors of less than 0.25 for all of the irradiated peaks, A–L; the saturation factor is defined as $(1 + \gamma^2H_2^2T_1T_2)^{-1}$,[15] and the value of 0.25 (i.e., $\gamma^2H_2^2T_1T_2=3$) is a maximum saturation factor that yields clear information on relative internuclear distances from time-dependent NOE experiments.[16] The time-dependent NOE experiments are needed when the spin diffusion mechanism is operating in the sample spin systems;[11,12,16] these results will be shown later. In the actual NOE difference spectra, the percentages of saturation of the irradiated peaks were calculated to be in the range of 80–95%, which indicates that the saturation factors are in the range of 0.2–0.05, that is, well within the above limit.

The most remarkable feature throughout these NOE difference spectra is that the saturation of any one of the peaks in a spectrum caused a decrease in intensities of all the other resonances in the spectrum including protons having intermolecular relationships with respect to the saturated protons, although the extent of intensity decrease of each peak varied with different irradiated positions. The loss of specificity thus observed in the steady-state NOE experiments for lecithin vesicles was an unexpected finding. Nevertheless, some specificity was observed in these steady-state NOE spectra. In spectrum 1l, irradiation of peak L caused a characteristic NOE (~20%) to peak D, and conversely, in spectrum 1d, irradiation of peak D caused a greater NOE to peak L than to any other peaks irradiated. These findings suggest that the phenothiazine ring is located near the α-methylenes of the fatty acid chains.[17]

In order to ascertain that these negative NOEs are not due to trivial receiver overload but to the long correlation times arising from anisotropic motions of lecithin and incorporated chlorpromazine molecules, we measured a similar set of steady-state NOEs for a nicotinamide–lecithin vesicle solution. Here, we can expect negative NOEs among lecithin protons and probably no NOE between protons of nicotinamide and those of lecithin since the nicotinamide molecule may be freely rotating in D_2O with a short correlation time of less than 10^{-10} s in contrast to the chlorpromazine incorporated into lecithin vesicles. This was indeed the case as shown in Figure 2, where, again, spectra 2a–l were obtained by irradiating the peaks labeled A–L in a normal ^1H NMR spectrum 2m. Evidently, no NOE can be seen between the protons of lecithin and those of nicotinamide, as expected; moreover, even positive NOEs (5–10%) were observed between protons within nicotinamide. Thus, it is unlikely that the receiver overload caused the negative NOEs shown in Figure 1. Interestingly, however, irradiation of the residual HDO signal at about 3.5 ppm caused very small (~0.5%) negative NOEs for the four resonances of nicotinamide. This result may be interpreted as being due to intermolecular interactions between HDO and a very small amount of nicotinamide both of which bind at the outer surface of a vesicle and reorient with nearly equal long correlation times of vesicles' Brownian motion. Interesting features were also found with steady-state NOE experiments performed 2 weeks after the nicotinamide had been dissolved into the lecithin vesicle solution. In these experiments, small but definite negative NOEs (0.3–0.5%) were observed from lecithin protons A–C, G, and I to the four resonances of nicotinamide. The marked negative NOEs from HDO also remained. These observations indicate that nicotinamide can also permeate into the lecithin bilayer very slowly.

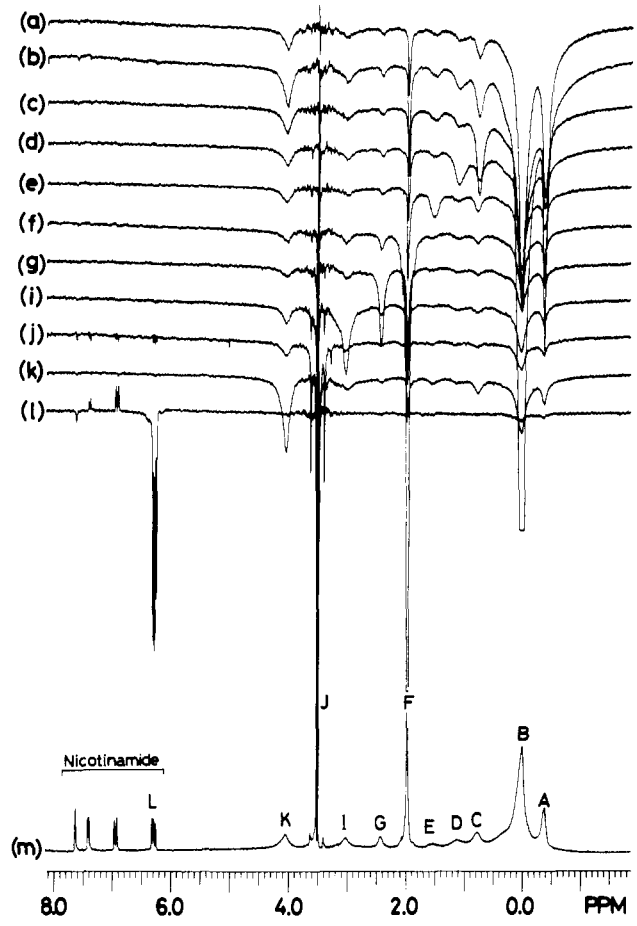

Figure 2. (a–l) Steady-state NOE difference spectra of nicotinamide (30 mmol dm^{-3})–lecithin vesicles (3%, w/v) in D_2O; (m) ^1H NMR spectrum of the nicotinamide–lecithin vesicles solution. In a–l, each spectrum was obtained by irradiating the corresponding peak labeled A–L in m.

Time-Dependent Nuclear Overhauser Effects. Although negativity of the observed NOEs can be understood as a consequence

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(17) It should be noted that peak D contains the resonance due to methylene protons ($-CH_2-$) of the chlorpromazine side chain. Contribution from this resonance to the negative enhancement of peak D in spectrum 1l is negligible since this resonance may be as broad as peak H ($N-CH_2-$ of chlorpromazine) as shown in spectrum 1m, even if an "NOE" is present between the phenothiazine ring protons and the side-chain protons. On the other hand, saturation of the side-chain methylenes ($-CH_2-$) may contribute to the negative NOE value of peak L to the extent of a few percent (the extent was estimated from a separate NOE experiment of chlorpromazine solution).

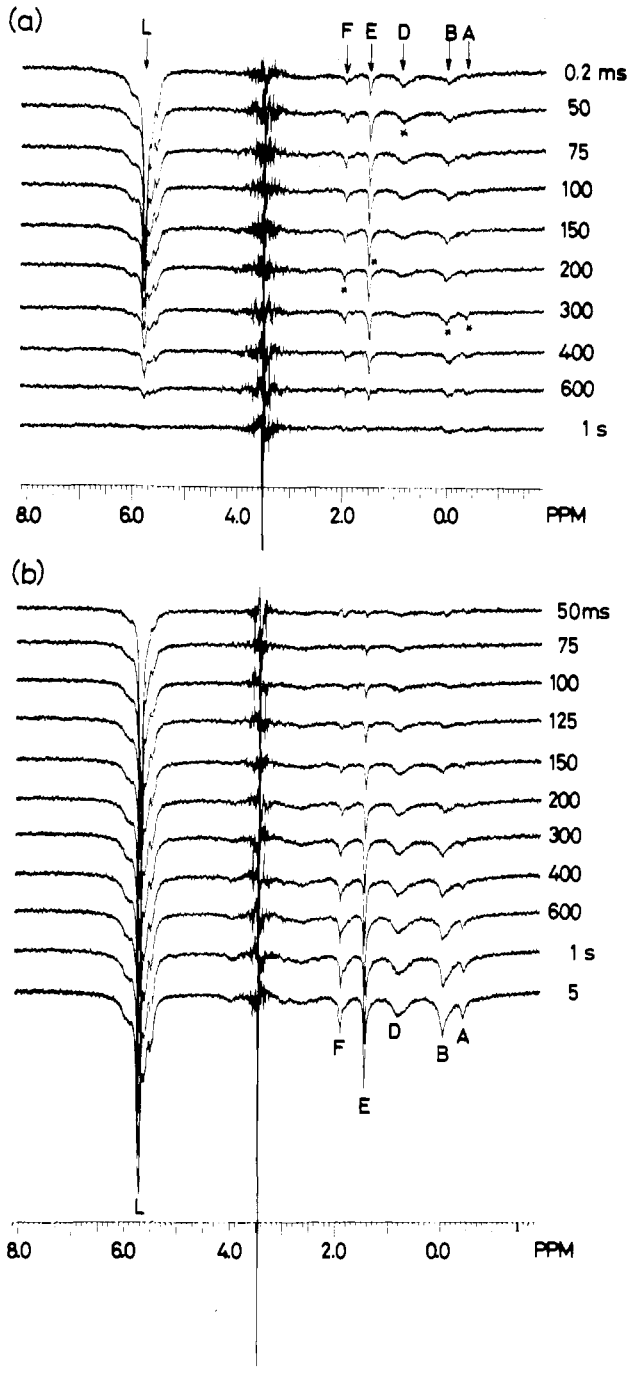


Figure 3. (a) Time-dependent NOE difference spectra of the chlorpromazine–lecithin vesicles solution by transient NOE experiments. The 180° inversion inpulse of 130-ms duration was applied to the phenothiazine ring proton resonances at about 5.8 ppm (peak L). The delay time between the 180° pulse and the sampling pulse is indicated on the right. Maximum growth of NOEs is indicated by an asterisk. (b) Time-dependent NOE difference spectra of the same solution as used in part a but obtained by truncated driven NOE experiments. Phenothiazine ring proton resonances at about 5.8 ppm (peak L) were irradiated at an H_2 level of $\gamma H_2/2\pi \sim 4$ Hz. The preirradiation time is indicated on the right.

of the dominant contributions from negative NOE enhancement due to long correlation times of interacting spins, the saturation transfer to protons distant from the irradiated site can only be explained by the concept of spin diffusion through lecithin and chlorpromazine molecules. If this is so, the time dependence of the NOEs should be measured to elucidate the spatial proximity between saturated protons and those showing negative NOEs since only the initial rate of buildup of NOE is related to the sixth power of the distance between them.[4a,10-12,16] Two methods have been reported for obtaining the time-dependent NOE spectra. One is

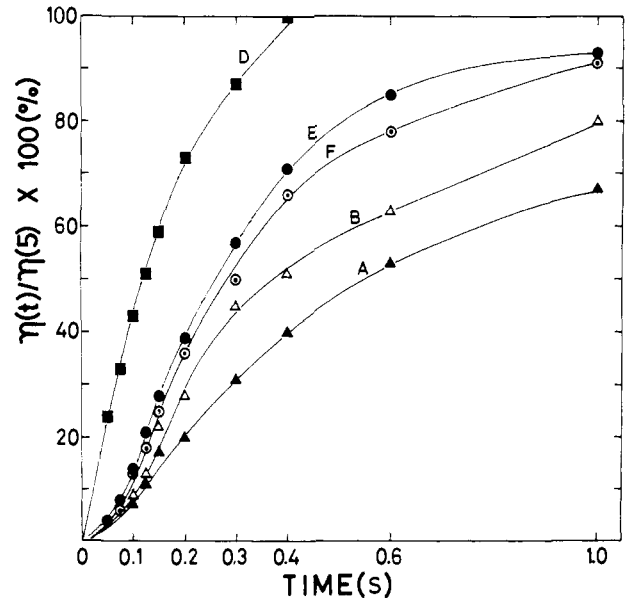


Figure 4. Plot of $\eta(t)/\eta(5) \times 100$ (%) vs. preirradiation time in the truncated driven NOE experiments of Figure 3b, where peak L was irradiated: (▲) peak A; (△) peak B; (■) peak D; (●) peak E; (⊙) peak F.

a transient NOE experiment[11b,12] and the other is a truncated driven NOE experiment.[11] Since these two methods have their own characteristics,[11,12] we initially employed both methods to see which is suitable for the present lecithin vesicle solution.

Figure 3 shows typical time-dependent NOE difference spectra thus obtained by the two methods. Both experiments were performed under the same attenuator setting that controls radio frequency irradiation power and with the same number of accumulations of FID. In the transient NOE experiment (a), a 180° inversion pulse with a pulse length of 130-ms was applied to the phenothiazine ring proton resonances (peak L) and the subsequent time development of NOEs was monitored as a difference spectrum by applying the same length of pulse to an appropriate off-resonance position. In the truncated driven NOE experiment (b), the same position was irradiated with an appropriate radio frequency power of 4 Hz ($\gamma H_2/2\pi$) and the resulting NOEs were monitored as a difference spectrum to an off-resonance irradiated spectrum and by varying the presaturation time as shown in the right-hand side of each spectrum. Although both sets of NOE difference spectra show clear time-dependent growth of NOEs, the relative magnitude of initial buildup rates of the NOEs can be more easily grasped from experiment a than from experiment b; the rate of growth of the NOE decreases in the order D $\gg$ E > F > B, A. Here, to show the ordering semiquantitatively, we used a sign of inequality. The maximum growth of NOE for each peak in experiment a was, however, two to three times smaller than that in experiment b, especially for slowly growing peaks such as A and B. This is because of spin–lattice relaxation in each resonance that is inherent in the transient method; therefore, no steady-state values of NOE are obtainable from method a. On the other hand, since NOEs in experiment b monotonously aim at reaching the steady-state values, it was easier to follow changes in peak intensity through different preirradiation times than to follow them in a similar manner in experiment a. Figure 4 shows the growth curves of each peak with variable preirradiation times. The ordinate indicates the percentage of growth of NOEs, that is, $\eta(t)/\eta(5) \times 100$ (%), where $\eta(t)$ is the intensity of a negative NOE at a preirradiation time t and $\eta(5)$ is its steady-state value. Clearly, curve D, where changes in the NOE at peak D were monitored, shows the fastest growth of NOE of the followed peaks. The overall ordering was D $\gg$ E > F > B > A in order from fastest to slowest buildup rates; this agrees well with the findings obtained from method a. Thus, it can be concluded that phenothiazine ring protons of chlorpromazine are located very near the α-methylenes in the fatty acid chains of lecithin. This con-

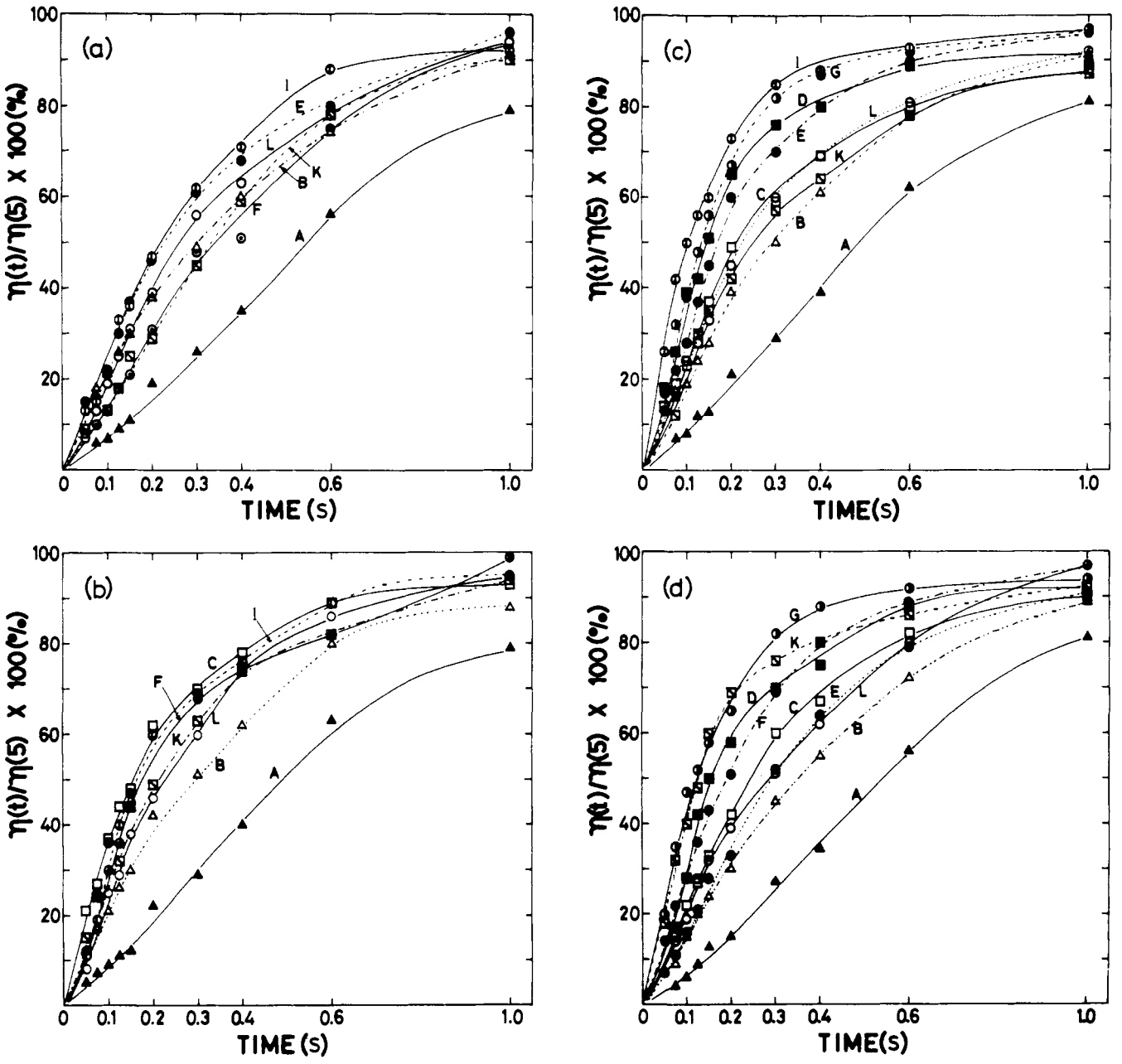


Figure 5. Plot of $\eta(t)/\eta(5) \times 100$ (%) vs. preirradiation time in the truncated driven NOE experiments. Irradiated peaks are (a) D, (b) E, (c) F, and (d) I: (▲) peak A; (△) peak B; (□) peak C; (■) peak D; (●) peak E; (⊙) peak F; (◑) peak G; (⊕) peak I; (⊠) peak K; (○) peak L.

Table II. Observed Orderings of Relative Rates of Growth of NOEs and Locations of Protons

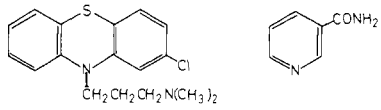
peak irrad (d)	assignment	observed ordering[a]	location of protons from the irradiated site	
			near	far
L	Phe(CP)[b,c]	D >> E > F > B > A	CH_2CO	$NH(CH_3)_2^+$(CP),[b] $N(CH_3)_3^+$, $(CH_2)_n$, CH_3
D	CH_2CO, $-CH_2-$(CP)[b]	I, E > L > B > F, K >> A	CH_2OCO, $NH(CH_3)_2^+$(CP),[b] Phe(CP)[b,c]	$N(CH_3)_3^+$, $CH{=}CH$, CH_3
E	$NH(CH_3)_2^+$(CP)[b] $=C-CH_2-C=$	C, I ≥ F ≥ K, L > B >> A	CH_2OP, $CH_2C{=}C$, $N(CH_3)_3^+$	$(CH_2)_n$, CH_3
F	$N(CH_3)_3^+$, CH_2-NH^+(CP)[b]	I > G > D > E > C, L > K > B >> A	CH_2OP, CH_2N^+	$CH{=}CH$, $(CH_2)_n$, CH_3
I	CH_2OCO, CH_2OP	G, K > D > F > C > E, L > B >> A	CH_2N^+, CHOCO, CH_2CO	$(CH_2)_n$, CH_3

[a] Decreasing rates of growth of NOEs are shown from left to right. [b] (CP): chlorpromazine. [c] Phe: phenothiazine ring protons.

clusion agrees well with the finding that signal D shifts upfield on addition of chlorpromazine probably due to a ring-current effect of the phenothiazine ring.

In order to ascertain whether the obtained ordering of buildup rates at short times can explain the spatial arrangements of the protons within lecithin vesicles which include chlorpromazine, we have applied method b to the other four peaks, D, E, F, and I. Growth curves of NOEs at variable preirradiation times are shown in Figure 5, and the orderings of buildup rates judged from these curves are summarized in Table II. These orderings were de-

termined by considering the curvature of the growth curves, especially in the time range of 0.1 to 0.4 s. We notice that some of these growth curves show similar buildup behavior. A possible explanation for this is that each irradiated (and naturally observing) peak is composed of more than two kinds of resonances (see peak assignments in Table I), and as a consequence the distance-dependent buildup rates lose their own specificity to some extent.[18] Therefore, taking into account the orderings, we simply classified the protons that showed negative NOEs into two groups, those locate near the irradiated site and those locate far from the site. The results are shown in Table II. Evidently, the correspondence between the irradiated site and the thus classified protons represents a reasonable spatial arrangement of a lecithin molecule, for example, such closely spaced relationships as CH_2CO/CH_2OCO, $N(CH_3)_3^+/CH_2OP,CH_2N^+$, and CH_2OP/CH_2N^+. These good correspondencies show not only the usefulness of the time-dependent NOE experiments but also the internal consistency of the presently adopted assignments for ^1H NMR resonances of lecithin. Of special interest regarding the assignments is that peak K includes the resonances due to both methine proton of glycerol (CHOCO) and olefinic protons (CH=CH); the latter is obvious from the expected chemical shifts. Only this assignment can explain well why peak K grows fast when peak I was irradiated. Interestingly, the overlapping resonances are also manifested in the steady-state NOE spectrum shown in figure 1. In spectrum 1i, where the methylene protons of glycerol are irradiated, the top of peak K is slightly displaced to high field compared to the others. Since the corresponding spctrum 2i does not show this upfield shift, the displacement can be ascribed to a ring-current effect of the phenothiazine ring of chlorpromazine. On the other hand, regarding the interaction site between chlorpromazine and lecithin, we notice that side-chain *N*-dimethyl protons of chlorpromazine (peak E) are located near the glycerol and choline methylenes (CH_2OP). Since the *N*-dimethyl group can be considered to be positively charged under our experimental conditions, it is reasonable to conclude that the positively charged *N*-dimethyl group of chlorpromazine is interacting with the negatively charged phosphate in the polar headgroup. A downfield shift of the *N*-dimethyl proton resonance (peak E) in Figure 1 (spectra 1o and 1m) may be caused by this interaction.



I II

Discussion

The spin diffusion process is known to transfer spin energy via spin–spin flip–flops between pairwise adjacent protons and from regions that are weakly coupled to the lattice to a heat sink coupling strongly to the lattice.[19] When the spin diffusion operates strongly in a spin system, no characteristic values are obtainable in either T_1 or steady-state NOE. The T_1 values are equalized to have an identical value and all NOEs become −1, thus losing selectivity.[10,20] Spin diffusion occurs under the conditions of $T_2 \ll T_1$ together with the presence of an appropriate heat sink relaxing efficiently with the lattice. The criterion, $T_2 \ll T_1$, is realized for molecules having long correlation times, τ_c, and at high external magnetic fields, since in these cases, the condition, $\omega\tau_c \gg 1$ (ω = the Larmor frequency of the proton) is satisfied and the transition probability W_0 (zero-quantum transition) becomes the dominating process in the dipole–dipole interactions which usually govern the thermal relaxation process of proton spin systems; thus, spin diffusion can usually be seen in solids[19,21] and proteins in solution.[10-12,22] Since the above criterion is fully satisfied in the case of unsonicated lecithin liposomes, it is claimed that spin diffusion occurs in these proton spin systems. A possible candidate of the heat sink has been shown to be the end groups[23] of the fatty acid chains or the choline group[24] at the polarhead end.[25] In the case of sonicated lecithin vesicles, on the other hand, the occurrence of the spin diffusion is denied[26] or considered to be not a dominant relaxation mechanism,[27] because T_1 values are not identical. Actually, the presently measured T_1 values of lecithin vesicles that contain chlorpromazine also show a slight variation in their magnitudes (Table I). The fact that the T_1 increases with increasing temperature[26] also complicates the decision whether or not spin diffusion can actually occur, because this implies that the effective correlation time (τ_{eff}) is in the situation $\omega\tau_{eff} \ll 1$. However, since in the sonicated lecithin $T_2 \ll T_1$,[26] we can consider a slow motion having a long correlation time τ_1 which satisfies the condition $\omega\tau_1 \gg 1$ within its intra- and/or intermolecular anisotropic motions. Then, it would naturally be expected that spin diffusion operates along the dipole–dipole vector reorienting with the time constant τ_1. Indeed, the nonselective steady-state NOEs observed presently can be considered to be a manifestation of the spin diffusion.[28] The different growth rates in the time-dependent NOE experiments Figures 4 and 5) lend further support to this view. In practice, however, the spin diffusion process may not be a domiant relaxation mechanism in the spin systems of sonicated lecithin vesicles, since T_1 values are not identical, and moreover, no marked changes in these values could be detected when portions of the spectrum, for example, the phenothiazine ring proton resonances (peak L), were irradiated (Table I). The enhancement of a spin–lattice relaxation rate (i.e., decrease of T_1) when a portion of the spectrum is irradiated has been discussed in detail by Akasaka for spin systems with strong spin diffusion.[22c] The present results show, however, that even if a spin system is in a state of "weak" spin diffusion, measurements of the initial rates of buildup of NOEs can give correct information on interproton distances. Thus, time-dependent NOE experiments make it feasible to elucidate the structure and the dynamics of membranes and their interactions with drugs.

In conclusion, spin diffusion occurs in sonicated lecithin vesicles as evidenced by the present "saturation transfer" experiments. This spin diffusion causes the steady-state NOEs within lecithin protons to be nonselective; thus, time dependence of the NOE should be measured to give growth rates at short times. The initial buildup rates show that the phenothiazine ring of chlorpromazine is located very near the α-methylenes of fatty acid chains and that the positively charged *N*-dimethyl group of the side chain interacts with the phosphate of the polarhead of lecithin.

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Registry No. Chlorpromazine, 50-53-3.

(18) Differences in mobility among the protons may also contribute to the complexities of the growth curves, since the cross-relaxation rate between the spins depends on a magnitude of the correlation time as well as on the interproton distance.

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