MAGNETIC RESONANCE STUDIES ON THE INTERACTION OF ANTIDEPRESSANTS WITH LIPID MODEL MEMBRANES

J. BERMEJO, A. BARBADILLO and F. TATO
Departments of Animal Physiology, Chemical Physics and Pharmacology, Santiago University, Spain

and

D. CHAPMAN
Department of Chemistry, Sheffield S3 7HF, England

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1. Introduction

The mechanism(s) by which some antidepressant-active drugs exert their specific action has remained a matter of speculation for several years.

To our knowledge, effects of imipramine-like drugs were assessed between others at synaptosome level, arguing then possible mechanisms for implication of these compounds in some aspects of membrane transport [1].

In a previous paper [2], we reported some experiments carried out in order to prove perturbations of liposome membrane properties induced by desipramine (DMI). However, the molecular mechanism of the process remained unclear. In the particular case of DMI, it was possible for us to obtain more information about the interaction by means of some physical techniques applied to the study of membrane microdynamics.

In the present study we have employed nitroxide labelled fatty acids with the paramagnetic center located at different positions in the chain, and a polar head labelled lecithin. Changes in the anisotropic motion of the labels incorporated into lipid model membranes produced by different concentrations of the drug are now assessed.

2. Experimental

1,2DPL was purchased from Koch-Light labs., hen egg-yolk lecithin (EYL) was extracted by the procedure of Singleton et al. [3]. Some of the spin-labels were a kind gift of Professor A. D. Keith, University of California, Berkeley. ESR spectra were recorded on a Varian E-3 (X band) spectrometer, using a dewared insert and copper-constantant thermocouple regulated N_2 gas flow.

^{13}C FT NMR spectra were obtained in a Jeol PFT 100 spectrometer operating at 25.152 MHz. The pulse with was 8 μsec and the computer delay time was 100 μsec. About 5000 free induction decays were apodised by means of an exponential window. Subsequently, these data points were Fourier transformed to give a real frequency domain spectrum.

3. Results

From investigations in nematic liquid crystals [4,5] several theories have been presented which extend the formalism given there to lyotropic mesophases of our kind [6].

Adscribing a coordinate system to the NO radical, such that z axis is in the direction of nitrogen $2p\pi$ orbital and the x one in the NO bond direction, it is possible to study the time averaged motion of this co-ordinate system with respect to the optic axis of the crystal, which in our case coincides with the normal of the bilayer plane; in terms of a traceless square matrix (the ordering matrix) which is for us axially symmetric, with elements

$$S_{ii} = (1/2)(<3\cos^2\theta - 1>) \quad i=1,2,3$$

where θ are the corresponding angles between each of the molecule fixed co-ordinates and the axis of the crystal.

It is also possible to describe the orientation by the use of a probability function expanded in real spherical harmonics $P(\theta,\varphi)$, which in our system can be reduced to $P(\theta) \alpha \exp[(-q/RT)\cos^2\theta]$, where θ is the angle between the long axis of the sticky molecule and z.

From these expressions and after some algebra we can write

$$S_{ii} = -1/2+3/2\,\frac{\int_0^{\Gamma} \cos^2\theta_i \exp[-(q_i/RT)\cos^2\theta_i]\sin\theta_i\, d\theta_i}{\int_0^{\Gamma} \exp[-(q_i/RT)\cos^2\theta_i]\sin\theta_i\, d\theta_i}$$

where q_i represents energy parameters giving the orientational energy of the label.

Table 1 gives several quantitative parameters which indicate the differences which appear when we add DMI.

In order to establish the elements of the diffusion tensor, we first have calculated the rotational correlation times of the labels by the use of the well known treatment of Waggoner et al. [7]. Rotational activation energies are also given in the table.

Fig.1 shows the apparent modifications of the ^{13}C NMR spectra of lecithin vesicles induced by DMI, which can be compared with the data extracted in another paper [2] which were obtained by the use of ^{1}H CW NMR, where methylene resonances showed an intensity increase accompanied by strong narrowing of the line. In the present case the chemical shift anisotropy resolution of the ^{13}C technique shows that the more affected resonances are due to the terminal methyl and to the last carbons of the chain.

A plot of the rotational correlation time of the 12 MeNS label versus DMI concentration is given in fig.2 and the shape of the curve can be readily compared with the one giving the DMI protection of hypotonic hemolysis of red blood cells given elsewhere [2].

4. Discussion

The investigation reported herein is concerned about the interaction of DMI with liposome membranes and an attempt to interpret the spectral modifications of several resonances due to the presence of the drug is now made.

Table 1
Order parameters and energetic data for the motion of spin label I (13.2) (4 NS)

Temp.		283	295	303	318	330	Erot – Erot (DMI)
Control	S_{33}	0.77	0.68	0.65	0.59	0.58	label.
	$-q_3$	4.088	3.197	3.027	2.729	2.769	
	S_{11}	−0.29	−0.34	−0.82	−0.27	−0.20	12 Me NS +2.502 Kc/mole
	$+q_1$	1.847	2.669	2.395	1.835	1.224	
DMI 5%	S_{33}	0.76	0.68	0.60	0.56	0.53	16 NS +3.210 Kc/mole
	$-q_3$	3.942	3.197	2.669	2.539	2.449	
	S_{11}	−0.32	−0.35	−0.30	−0.24	−0.16	
	$+q_1$	2.236	2.886	2.106	1.525	0.922	PPTC*NS–4.183 Kc/mole
DMI 10%	S_{33}	0.79	0.69	0.60	0.53	0.51	
	$-q_3$	4.424	3.289	2.669	2.359	2.339	* di Palmitoil phosphatidyl tempocholine
	S_{11}	0.37	0.36	0.29	0.21	0.15	Samples were prepared adding a 0.08
	$+q_1$	3.226	3.087	1.978	1.260	0.852	DMI to lipid molar ratio.
DMI 20%	S_{33}	0.80	0.75	0.59	0.53	0.50	
	$-q_3$	4.617	3.966	2.599	2.359	2.279	
	S_{11}	−0.41	−0.40	−0.27	−0.18	−0.10	
	$+q_1$	4.666	4.373	1.748	1.028	0.535	

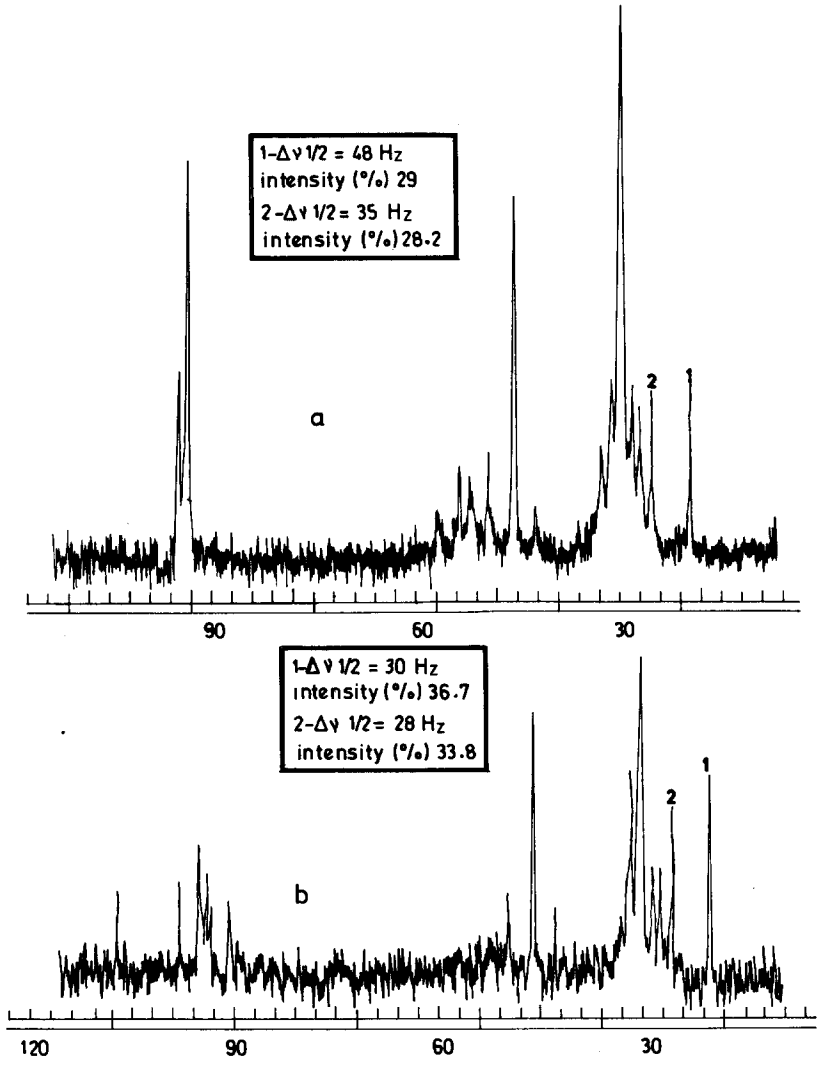

Fig.1. 13c FT NMR spectra of EYL (a) and EYL with 5% DMI added (b)

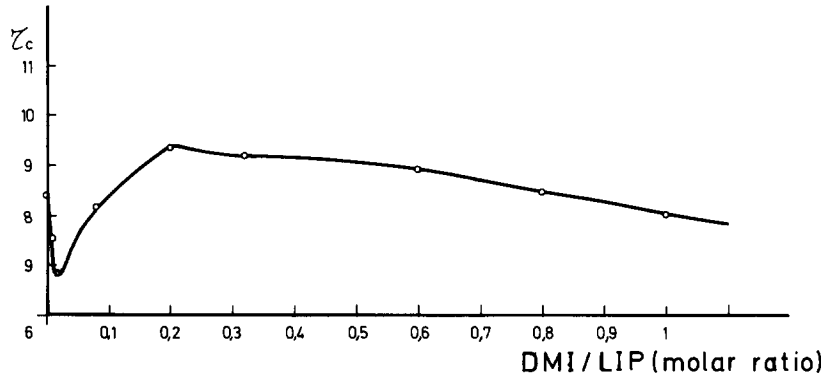

Fig.2. DMI concentration dependence of rotational correlation time (EYL (12 - NSMe) room temperature

Table 2
a: Computed from conformational data
b: Computed by means of the partition function given in ref [10] Assumed bond orientation potential, (E_r=1500 cal/mole)

T	Pt	Pg	Sa	Sb	$\bar{r}_3$	
283	0.98	0.02	0.111		23	Control
283	0.99	0.01	0.060		21	DMI
303	0.94	0.06	0.268	0.88	28.5	Control
303	0.91	0.09	0.364	0.92	31.5	DMI
318	0.88	0.12	1.028	0.96	31.5	Control
318	0.82	0.18	1.823	1.02	34.5	DMI
330	0.83	0.17	1.754	1.12	32	Control
330	0.79	0.21	2.016	1.16	35.3	DMI

In a publication, Cater et al. [8] reported mainly calorimetric evidence (DSC) of modifications in the physical state of the lipid produced by a large series of compounds, assuming in the case of DMI some modification to be responsible of the strong shift in the transition temperature (T_c). With the parameters obtained here it is possible to make a measure of the tilt and spread angles of the chains, and the differences in tilting caused by DMI. On the other hand application of the rotational isomeric model with [9] or without chain interaction [10], percentages of carbon–carbon bonds in *cis* and *trans* configuration are derived, from which estimations of the configurational entropy are calculated, and compared with the ones calculated using the partition function given elsewhere [10].

At this point and making some recapitulation of the data given above, we can postulate some remarks about a possible quantitative molecular model of the interaction. First, in systems above or near the phase transition, DMI exerts a disordering effect of the bilayer by means of some kind of membrane expansion which implies an increase in the percentage of gauche bonds possibly due to an increase in the area occupied by each molecule. If the lipid is well below the transition; DMI is now hindering the chain motion as order parameters at temperatures in this range demonstrate. Second, if the lipid is above the transition like EYL at room temperature, the response of the bilayer to different concentrations of the drug is not linear. And third, according to fluorescence data given in [2], the proper location of the DMI molecule in the bilayer can be accomplished if one assumes that the iminodibenzyl part is located well in the hydrocarbon phase and the short chain near the polar groups exerting the amino portion a strong inmobilization of them like the E_{rot} of the nitroxide labelled phospholipid and the broadening of the choline methyl resonance indicates. So, with this type of molecular assemblage a possible explanation of DMI effects on permeability can be argued.

A detailed report about the stoichiometry and cooperativity of the system will be published in future, hoping to obtain a more precise information about the interaction.

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