STUDIES OF CHROMOPHORES IN MODEL MEMBRANES BY POLARIZED LIGHT-ABSORPTION SPECTROSCOPY

THE ORIENTATION AND BINDING OF TETRACAINE AND PROCAINE

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ABSTRACT   A method for studying the orientation and binding of chromophores in macroscopically aligned membranes by polarized light absorption spectroscopy is described. Here tetracaine and procaine solubilized in a lamellar phase of octanoyl-1-glyceride (monooctanoin) and water have been investigated. Tetracaine is found to be located in the lipid region with a preferential orientation of the molecular long axis parallel to the hydrocarbon chains. The orientation of procaine, mainly residing in the water region, is very small.

INTRODUCTION

There has long been a great interest in the physicochemical properties of membrane-active drugs in lipid bilayers. This pertains in particular to local anesthetics, which are drugs that reversibly block the action potential of the nerve or muscle cell without appreciably affecting the resting membrane potential of the cell. Recent reviews on the membrane actions have been written by Seeman (1) and Papahadjopolous (2), and a book edited by Fink (3) is available. Because local anesthetics like tetracaine and procaine have surface active properties they are thought to function through interactions with the membrane lipids. Such a lipoid theory was originally proposed by Overton (4) and Meyer (5), emphasizing the predominant role of lipid solubility of the noncharged form of the anesthetic. Skou (6) also demonstrated a correlation between the potency of local anesthetics in blocking nerve conduction and their penetration into lipid monolayers.

Previously, nuclear magnetic resonance methods (NMR) have also been used to investigate the interaction of local anesthetics with membrane lipids, but contradictory results have been reported since Hauser et al. (7) concluded from their findings that no interaction occurs between local anesthetics and zwitterionic phosphatidylcholine in the absence of a net negative charge. The interaction was therefore thought to be mainly electrostatic. Fernández and Cebrón (8), on the other hand, showed that tetracaine interacts hydrophobically with zwitterionic lecithin, whereas procaine does not.

To get further insight into the interaction of anesthetics with lipid membranes we used a polarized absorption spectroscopical method (linear dichroism) to investigate the binding and molecular orientation of tetracaine and procaine in model membranes composed of a noncharged lipid, monooctanoin. Light spectroscopy is particularly useful for such studies, since almost all anesthetics contain rather strong chromophores. The molecular orientation of

the chromophore in the membrane can be determined from measurements of the linear dichroism on a macroscopically aligned sample. In a recent review (9), the theoretical treatment of polarized light spectroscopy on membrane systems has been given together with a critical discussion of the methods used to obtain the molecular orientation.

METHOD

The absorbance (A) that arises from the interaction of the electronic dipole transition moment and the electric field vector of weak electromagnetic radiation is given by (9)

$$A = k \, E^L \, \langle P^L \rangle \, E^{L,t}, \tag{1}$$

where E^L is a (1×3) matrix representation of the polarization of light and $\langle P^L \rangle$ is the absorptivity tensor represented by a (3×3) matrix. For macroscopically anisotropic systems the matrix elements of $\langle P^L \rangle$ contain linear combinations of the order parameters (9). The order parameters are ensemble-averaged Wigner rotation matrix elements denoted as $\langle D_{nm}^{(2)*}(\alpha, \beta, \gamma) \rangle$ (10). α, β, and γ are the Eulerian angles.

Consider a system in which a mole fraction, p, is in a uniaxially anisotropic site while the remaining fraction, $1\text{-}p$, is in an isotropic site. The normalized distribution density function, f', of the system can then be written

$$f'(\beta, \gamma) = (1\text{-}p) \frac{1}{8\pi^2} + p f(\beta, \gamma), \tag{2}$$

where $f(\beta, \gamma)$ is the uniaxially symmetric distribution density function of the anisotropic site. Applying this distribution density, one obtains

$$\int_{\alpha,\beta,\gamma} f'(\beta, \gamma) \, D_{nm}^{(2)*}(\alpha, \beta, \gamma) \sin \beta d\alpha d\beta d\gamma$$

$$= p \int_{\beta,\gamma} f(\beta, \gamma) \, D_{om}^{(2)*}(\beta, \gamma) \sin \beta d\beta d\gamma \equiv p \langle D_{om}^{(2)*}(\beta, \gamma) \rangle. \tag{3}$$

For such a system the absorptivity tensor (9) is

$$\langle P^L \rangle =$$

$$\begin{pmatrix} \frac{1}{3} Tr\tilde{P} - \frac{1}{3} \sum_{n=-2}^{2} p \langle D_{on}^{(2)}(\beta, \gamma)^* P_n^M & 0 & 0 \\ 0 & \frac{1}{3} Tr\tilde{P} - \frac{1}{3} \sum_{n=-2}^{2} p \langle D_{on}^{(2)}(\beta, \gamma)^* \rangle P_n^M & 0 \\ 0 & 0 & \frac{1}{3} Tr\tilde{P} + \frac{2}{3} \sum_{n=-2}^{2} p \langle D_{on}^{(2)}(\beta, \gamma)^* \rangle P_n^M \end{pmatrix} \tag{4}$$

P_n^M are the irreducible spherical tensor components of the molecular absorptivity tensor. The absorbances with the polarizations of light perpendicular ($A_\perp$) and at an angle (A_ω) with the director or the laboratory Z-axis (see Fig. 1) yield

$$A_\perp = k(0 \; 1 \; 0) \langle P^L \rangle (0 \; 1 \; 0)^t = k \left(\frac{1}{3} Tr\tilde{P} - \frac{1}{3} \sum_n p \langle D_{on}^{(2)*}(\beta, \gamma) \rangle P_n^M \right), \tag{5}$$

$$A_\omega = k(-\sin\omega \; 0 \; \cos\omega) \langle P^L \rangle (-\sin\omega \; 0 \; \cos\omega)^t$$

$$= k \left(\sum_n p \langle D_{on}^{(2)*}(\beta, \gamma) \rangle P_n^M \cos^2\omega + \frac{1}{3} Tr\tilde{P} - \frac{1}{3} \sum_n p \langle D_{on}^{(2)*}(\beta, \gamma) \rangle P_n^M \right). \tag{6}$$
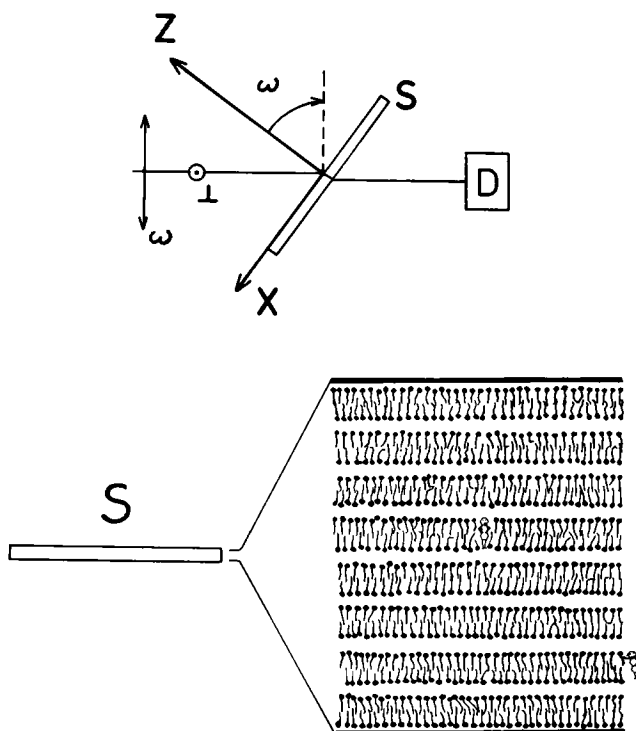
FIGURE 1 Polarized absorption measurement. Light with the polarization direction either ω or $\perp$ impinges on an aligned lamellar sample (S). The transmitted light is detected at D.

The molecular orientation is obtained from the linear dichroism, $LD \equiv A_\omega - A_\perp$, and $A_\perp$, according to Eq. 7:

$$\frac{LD}{A_\perp} = \frac{3 \sum_n \langle D_{on}^{(2)*}(\beta, \gamma) \rangle P_n^M \, p \cos^2\omega}{Tr\tilde{P} - \sum_n \langle D_{on}^{(2)*}(\beta, \gamma) \rangle P_n^M \cdot p}. \tag{7}$$

If the molecular z-axis is chosen along the transition moment, n is equal to zero in Eq. 7. Thus,

$$\frac{LD}{A_\perp} = \frac{3 \langle D_{00}^{(2)}(\beta)^* \rangle p \cos^2\omega}{1 - \langle D_{00}^{(2)*}(\beta) \rangle p} = \frac{3pS}{1 - pS} \cos^2\omega, \tag{8}$$

where $S = \langle D_{00}^{(2)}(\beta) \rangle$ is the more familiar order parameter introduced by Saupe (11).

EXPERIMENTAL

Absorption spectra were recorded on a Varian Cary 219 absorption spectrometer (Varian Associates, Inc., Palo Alto, Calif.). The linear dichroism, LD, was measured on a home-built apparatus utilizing a Jobin Yvon HRS2 monochromator and a Jobin Yvon photoelastic modulator.

Monooctanoin was purchased from Syntestjänst, Lund University; procaine and tetracaine were bought from Sigma Chemical Co., St. Louis, Mo. The samples were macroscopically aligned between quartz plates according to a procedure previously reported (12, 13). All experiments were performed at ~pH 6.
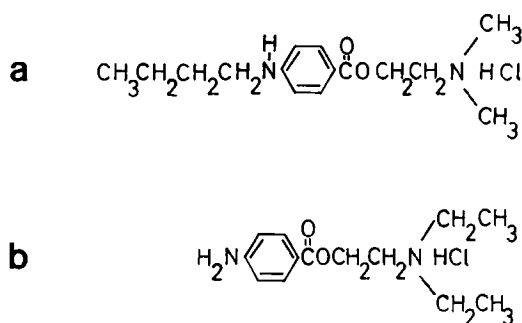
FIGURE 2 Structure formulas of (a) tetracaine and (b) procaine.

RESULTS AND DISCUSSION

Tetracaine (hydrochloride) in water absorbs light with a maximum at ~310 nm, with a molar absorptivity determined to be 2.3×10^4 mol^{-1} dm^{3}cm^{-1}. It can be assumed that this absorption is due to a $\pi^* \leftarrow \pi$ transition polarized in the plane of the phenyl ring and directed along the parasubstitution of the ring (Fig. 2). The $\pi^* \leftarrow \pi$ assumption is supported by the red shift obtained for tetracaine (uncharged, free base) when dissolved in solvents with increasing polarity as shown in Table I, where λ_{max} of the drug in hexane, ethanol, and water is given.

Knowing the direction of the transition dipole in the molecule, one can study the orientation of tetracaine in a macroscopically aligned sample by polarized absorption spectroscopy. Such a method has been used here to study the orientation of the drug solubilized in a model membrane composed of octanoyl-1-glyceride, monooctanoin, and water. The absorption of light was detected with the macroscopically aligned sample at an angle of $\pi/4$ between the normal of the quartz plates and the direction of propagation of light as depicted in Fig. 1. A detailed discussion of this technique has been reported previously (9). The results obtained are shown in Fig. 3, where $LD/A_\perp$ is plotted as a function of the molar ratio between monooctanoin and tetracaine. It is clear from this Figure that $LD/A_\perp$ for the charged tetracaine is constant at all concentrations varying over more than one order of magnitude. On the other hand, for the tetracaine free base, $LD/A_\perp$ is lower than that of tetracaine at high concentration, but it approaches the constant value obtained for tetracaine at lower concentra-

TABLE I
ABSORPTION MAXIMA OF TETRACAINE IN THREE DIFFERENT SOLVENTS

Solvent	Chromophore	$\lambda_{max} \pm 1$ nm
n-Hexane	Tetracaine	
	free base	292
	Tetracaine	—*
Ethanol	Tetracaine	
	free base	308
	Tetracaine	311
Water	Tetracaine	
	free base	309
	Tetracaine	311

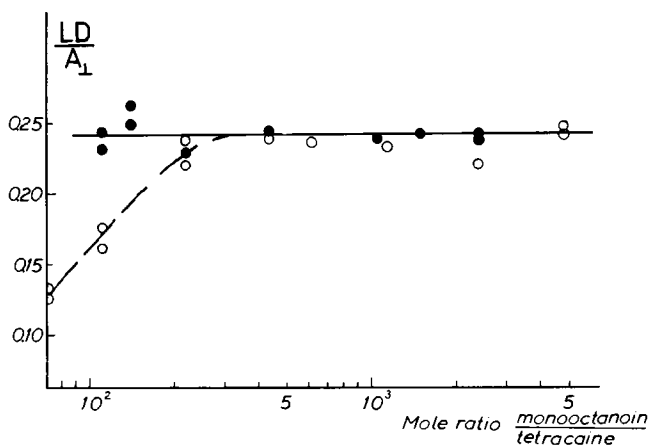*Solubility $<10^{-6}$ mol dm^{-3} and could therefore not be observed.

FIGURE 3 The ratio between the linear dichroism, LD, and the absorption perpendicular to the quartz plates, $A_\perp$, as a function of the molar ratio between monooctanoin and tetracaine (●), and as a function of the molar ratio between monooctanoin and tetracaine free base (○). The temperature was 22°C.

tions of the drug. These experimental findings can be interpreted by adopting the two-site model considered in the Method section with the following two sites: (a) molecules residing in an anisotropic environment within the lipid bilayer, having an order parameter S; and (b) molecules in the water layer subjected to an isotropic orientational distribution giving rise to an order parameter equal to zero. The mole fraction of molecules in the anisotropic site is p. The order parameter can then be obtained from

$$\frac{LD}{A_\perp} = 3pS(1 - pS)^{-1} n^{-2}(1 - n^{-2}\cos^2\omega)^{-1/2}\cos^2\omega, \qquad (9)$$

where also n, the refractive index of the lamellar sample, has been taken into account (14). Since $n = 1.5$ (15) and $\omega = \pi/4$, the constant value of the apparent order parameter, pS, of tetracaine can be calculated to be 0.24 ± 0.02. The lowest value of pS for tetracaine free base is equal to 0.15. The positive order parameter ($p > 0$) for both tetracaine and tetracaine free base implies that the long axes of the molecules are preferentially oriented parallel to the hydrocarbon chains.

Since the tetracaine molecule is amphiphilic (Fig. 2), with a rather long hydrophobic part, a constant pS over a wide concentration range strongly indicates that the drug is solubilized within the lipid bilayer. This is further supported by the rather high value of $pS = 0.24$. In previous studies (9) we have found that the order parameters of long chromophores solubilized in monooctanoin are in general $\sim0.1–0.3$; e.g., for retinal $S = 0.28$. These observations taken together strongly indicate that most of the tetracaine molecules are located in the lipid bilayer; i.e., p is close to unity. This interpretation is further supported by the findings obtained for the free base. For this molecule $LD/A_\perp$ is the same as for tetracaine at low concentrations but decreases at higher concentrations. This means that either p and/or S diminish with increasing free-base content. Since this molecule is hydrophobic it may be located also in the interior of the membrane, where the molecular ordering is lower, as has been shown by deuteron NMR studies (16). With increasing concentration of this drug it may therefore be

TABLE II
ABSORPTION MAXIMA OF TETRACAINE SOLUBILIZED TO VARIOUS AMOUNTS IN THE
LAMELLAR PHASE COMPOSED OF MONOOCTANOIN AND WATER

C_8-monoglyceride/anesthetic	$\lambda_{max} \pm 1$ nm	
	Tetracaine free base	Tetracaine
Molar ratio		
$4.33 \cdot 10^3$	313	—
$2.16 \cdot 10^3$	312	310
$1.62 \cdot 10^3$	—	310
$1.08 \cdot 10^3$	312	310
$4.32 \cdot 10^2$	312	310
$2.16 \cdot 10^2$	311	310
$1.44 \cdot 10^2$	—	310
$1.08 \cdot 10^2$	310	310
$7.20 \cdot 10$	309	—

expected that an increasing amount of the molecules have to be in the middle of the lipid bilayer. Hence, the most probable explanation of the decrease observed in $LD/A_\perp$ is due to the lower molecular ordering of molecules at the center of the bilayer. This conclusion is supported by the following experimental finding shown in Table II. An increase in the content of tetracaine free base leads to a shift of the absorption maximum to a shorter wavelength, whereas for tetracaine the λ_{max} does not vary with the concentration. Thus, at higher concentrations higher amounts of tetracaine free base will be located in a more hydrophobic milieu (Table I). For tetracaine, on the other hand, the solubilization site will not change with concentration.

For procaine the LD was found to be very small. The order parameter calculated after correction for multiple reflection (15) was negative and very small, in the order of 10^{-2}. The observation of a slight decrease in the order parameter with increasing procaine content indicates that procaine is mainly located in the water layer.

CONCLUSIONS

It has been found that the drug tetracaine is completely solubilized with an order parameter equal to 0.24 in the lipid membrane. Procaine, on the other hand, was found to reside in the water layer with a very small order parameter. The method used here could be generally applied for studies of the binding and orientation of chromophoric molecules in macroscopically aligned samples.

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