STRUCTURAL ANALYSIS OF DRUG MOLECULES IN BIOLOGICAL MEMBRANES

LEO G. HERBETTE, DAVID W. CHESTER, AND DAVID G. RHODES
Departments of Medicine and Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06032; and Department of Biology, Brookhaven National Laboratory, Upton, New York 11973

1,4-dihydropyridine (DHP) calcium channel antagonists bind with very high affinity (0.1–5 nM) to specific membrane-associated receptors in cardiac sarcolemma (1). Several characteristics of the canine cardiac sarcolemmal membrane and drugs that bind to receptors in this membrane (2, 3) suggest that it is appropriate to consider a two-stage binding mechanism that involves partitioning of the drug molecule into the lipid bilayer, where it may become oriented and then diffuse laterally to a receptor binding site.

To define the molecular properties of cardiovascular drugs in membranes, it is helpful to have a molecular picture of the drug directly determined in its natural environment. We have used x-ray and neutron diffraction techniques with both model and native biological membranes to determine the position, orientation, and conformation of a β adrenergic antagonist (propranolol) and a DHP calcium channel antagonist (nimodipine) in a membrane bilayer. These results are being combined with previous studies to establish a molecular mechanism for the binding of drugs to their receptors in the heart.

Several previous observations from cardiac sarcolemmal membrane suggest that the membrane bilayer is important in drug binding to cardiac receptors. Colvin et al. (2) have demonstrated that receptor site densities (sites/μm^2) in sarcolemma are quite low for some ligands: ouabain, 330; quinuclidinyl benzilate, 5; dihydroalprenolol, 2; nitrendipine, 1. Herbette et al. (3) have demonstrated, using x-ray diffraction, that the structure of this membrane resembles that of a pure lipid bilayer, as might be expected from the high lipid-to-protein ratio (3 μM phospholipid/mg protein). X-ray and neutron diffraction have been used to determine the precise location of propranolol (4) and the DHP Bay P 8857 in model membranes. The naphthalene moiety of propranolol and the five-substituent side chain of Bay P 8857 are both located within the first few methylene segments of the fatty acyl chains in these bilayers. That partition coefficients determined for these antagonists in the membrane[1] Table I) are significantly higher than those obtained in octanol/buffer indicates the necessity to understand drug-membrane interaction at the molecular level. In addition, Bay P 8857 and nimodipine had intrinsic binding rates of 3×10^7 and 1×10^7 $(Ms)^{-1}$, at 25°C. Nonspecific binding of these drugs to membranes without specific receptors appeared to be at least 1,000 times faster than specific binding. These experimental rates of binding can now be evaluated in light of recent theoretical diffusion-limited rates in which drug binding to sarcolemmal receptors via the membrane bilayer is at least 1,000 times faster than an aqueous pathway approach (5). We describe here some structural measurements undertaken to explore further the possibility that partitioning of these drugs into the lipid bilayer matrix of receptor containing membranes is necessary for binding of these drugs to cardiac membrane receptors.

RESULTS AND DISCUSSION

X-ray crystallography was used to determine the crystal structures of propranolol and nimodipine (Fig. 1). The propranolol molecule is nearly planar with the charged amine group 5.5 Å from the center of mass of the naphthalene moiety. The crystal structure of nimodipine was similar to that of other DHP calcium channel antagonists (6) with the planes of the nitrophenyl and pyridine rings approximately perpendicular and the pyridine ring slightly puckered.

Neutron diffraction studies showed that the center of

TABLE I
PARTITION COEFFICIENTS

Drug*	Biological Membranes	Octanol/Buffer‡
Bay P 8857	125,000	42
Nisoldipine	6,000	38
Nimodipine	5,000	730
Propranolol	200	18
Acetylcholine	32	0.003
Timolol	16	0.7
Ethanol	3	0.6

*Bay P 8857, Nisoldipine and Nimodipine are DHP calcium channel antagonists; Propranolol and Timolol are beta adrenergic antagonists.
‡Buffer was 150 mM NaCl, 10 mM Tris/Cl, pH 7.2.

[1]Membrane partition coefficients were determined in sarcoplasmic reticulum in order to reflect only nonspecific drug binding.

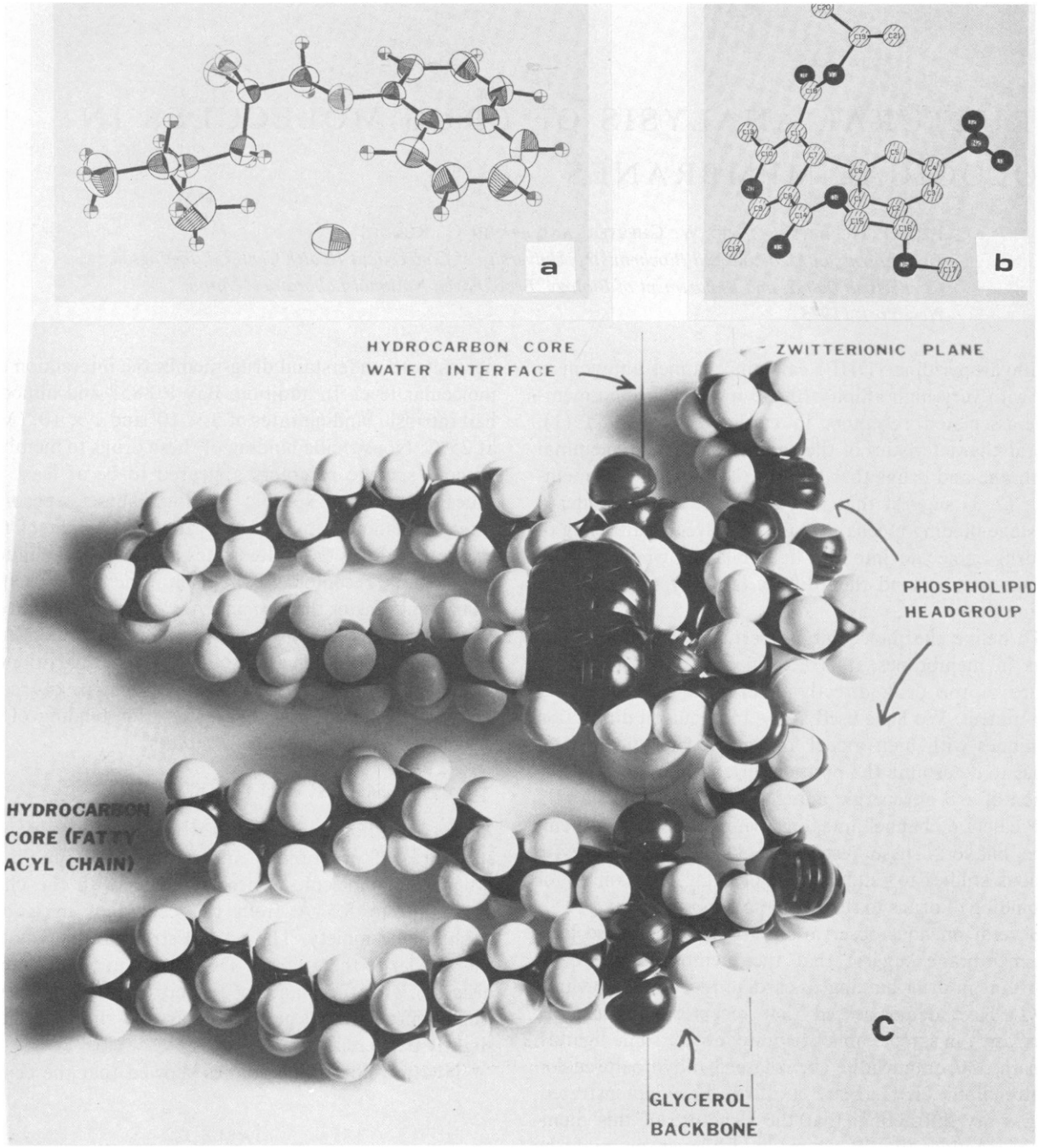

FIGURE 1 Crystal structure of (*a*) propranolol and (*b*) nimodipine as determined by x-ray crystallographic methods. (*c*) Placement of the crystal structure of propranolol in a membrane bilayer as suggested by neutron diffraction. Please refer to the color figure section at the back of this book.

mass of the naphthalene moiety of propranolol is located ~10 Å from the bilayer surface, in the region of the first few methylene segments of the fatty acyl chains. Because water penetrates the headgroup region only up to the glycerol backbone, the naphthalene moiety of propranolol is in a region of the bilayer where water is excluded. With the crystal structure of propranolol superimposed on the structure of a lipid bilayer, and positioned as determined by neutron diffraction, the charged amine lies within the phospholipid headgroup region, where it could be involved in ionic interactions.

Neutron diffraction was used to determine the nonspecific binding location of deuterated nimodipine (pyridine: 2,6-CD_3) in the sarcoplasmic reticulum. The difference profile demonstrated that nimodipine was located in the protein knob region and at both water/hydrocarbon core interfaces of the bilayer, with more drug appearing in the inner monolayer. Its position near the water/hydrocarbon

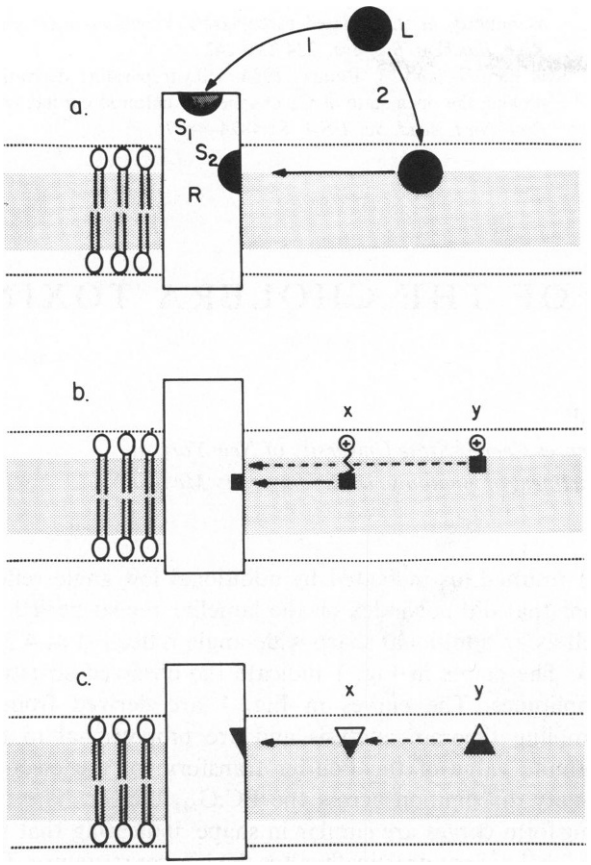

FIGURE 2 (*a*) A drug *L* may reach a binding site S_1 or S_2 on a membrane associated receptor protein (*R*) by *1* direct diffusion through the aqueous solvent or by *2* partitioning into the lipid bilayer and then diffusing laterally to the active site. (*b*) The highly ordered strucutre of the lipid bilayer may restrict membrane soluble drugs to a particular depth of penetration into the bilayer (drug *X* would be a reactive molecule since it is positioned at the proper depth of penetration for optimal reaction with a protein receptor site, drug *Y* would be inactive). (*c*) Bilayer constraints on the orientation of drug molecules relative to the active site might also affect their activity (drug *X* would be active, drug *Y* would be inactive).

core interface in the membrane bilayer is similar to that of propranolol's naphthalene ring. Although the location of the nitrophenyl ring of nimodipine has not been determined in the membrane bilayer, the crystal structure (Fig. 1 *b*) suggests that it would be positioned within the hydrocarbon core region of the bilayer. Because the nimodipine was added externally to sealed sarcoplasmic reticulum vesicles, this neutron diffraction experiment also demonstrated that these drugs can flip-flop, that is, diffuse between monolayers of the same membrane bilayer. That more nimodipine was found in the inner monolayer is consistent with the observation that the inner monolayer contains ~8% more lipid than the outer monolayer (7). These results demonstrate that (*a*) these drugs bind nonspecifically to both protein and lipid components of the membrane; (*b*) the molar ratio of drug:lipid is the same for both monolayers; and (*c*) these drug substances can cross the membrane bilayer of biological membranes.

CONCLUSIONS

X-ray and neutron diffraction together can define the precise location, conformation, and orientation of drug molecules in model and biological membranes and, when combined with other techniques, can provide valuable information for elucidating the mechanism of drug-receptor interactions. These techniques were used in the present study to determine whether the nonspecific interaction of certain cardiovascular drugs with the membrane bilayer is a component of the pathway by which these drugs reach specific protein receptors. Although the model needs further testing, it is supported by recent patch-clamp studies carried out with cultured cardiac cells (8). Knowledge of the structure of the drug when it nonspecifically incorporates into the membrane bilayer and when it binds to its receptor may help us to understand the molecular basis for how these drugs function to alleviate a particular cardiovascular abnormality. This understanding may help establish essential molecular criteria (see Fig. 2) for the design of new drugs that are more potent in action, more selective for binding to specific receptors, and that function with fewer side effects.

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X-RAY DIFFRACTION STUDIES OF THE CHOLERA TOXIN RECEPTOR, G_{M1}.

ROBERT V. MCDANIEL* AND THOMAS J. MCINTOSH‡
**Department of Physiology and Biophysics, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, New York 11794; and ‡Department of Anatomy, Duke University Medical Center, Durham, North Carolina 27710*

Gangliosides are anionic glycolipids that are found in the outer monolayer of many plasma membranes. In particular, the polar head group of ganglioside G_{M1} plays an important role in human physiology as it is the receptor for cholera toxin (1) and it regulates growth factor receptors (2). Gangliosides will not form bilayers by themselves, presumably because their head groups are large (3–5). However, multilamellar liposomes formed from mixtures of phosphatidylcholine (PC) and G_{M1} (4) have been used as models of the electrokinetic properties of human erythrocyte membranes (6). In this report, the structure of PC:G_{M1} liposomes is analyzed by x-ray diffraction techniques to determine the distance by which the polar head group of G_{M1} extends from the bilayer surface. We label the sialic acid moiety of G_{M1} with europium (Eu) (5) and use difference profiles to determine the distance between the fixed charge on G_{M1} and the phosphate group of PC.

MATERIALS AND METHODS

We formed bilayers from a 7:3 mol:mol mixture of 1-palmitoyl-2-oleyl phosphatidylcholine (PC) with G_{M1}. PC was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL). G_{M1} (>90% by TLC) was prepared from upper Folch extract of bovine brain (7). Eu-labeled G_{M1} was prepared by adding 1 mol europium chloride/mol G_{M1} in methanol:water 33:1, followed by evaporation of solvent. Europium chloride (99.9%) was obtained from Alfa Inorganics, Inc. (Beverly, MA). Swelling experiments were performed by gravimetrically adding dry lipid to increasing amounts of aqueous 0.1 M NaCl, pH 7.5. The x-ray patterns were recorded and analyzed by standard techniques, as described previously (8).

RESULTS

For a range of water contents of ~15–50 wt %, each diffraction pattern contained five or six orders of a single lamellar repeat period and a broad wide-angle band at 4.6 Å, indicating a single multilamellar phase of liquid crystalline bilayers. We obtained lamellar repeat periods of 62–70 Å for unlabelled bilayers and 62–78 Å for Eu-labelled bilayers. At repeat periods larger than this range, only two or three lamellar diffraction orders were recorded. At repeat periods smaller than this range, phase separation (4) resulted, as indicated by additional low-angle reflections that did not index on the lamellar repeat period, as well as an additional sharp wide-angle reflection at 4.2 Å (3). The points in Fig. 1 indicate the observed structure amplitudes. The curves in Fig. 1 are derived from a sampling theorem analysis and are proportional to the absolute value of the Fourier transform of the electron density distribution across the PC:G_{M1} bilayer. The two transform curves are similar in shape, indicating that the Eu label did not drastically alter the bilayer structure. The correct phase angle, either π or 0, was assigned to each region of the transforms by using the sampling theorem (8). The resulting electron density profiles are shown in Fig. 2. The top profile is of a bilayer labelled with Eu and the middle profile is of an unlabelled bilayer. The Eu difference profile (bottom of Fig. 2) was obtained by subtracting the scaled unlabelled profile from the labelled profile, with a scaling factor that assumes no penetration of Eu ions into the hydrocarbon region of the bilayer (5). The

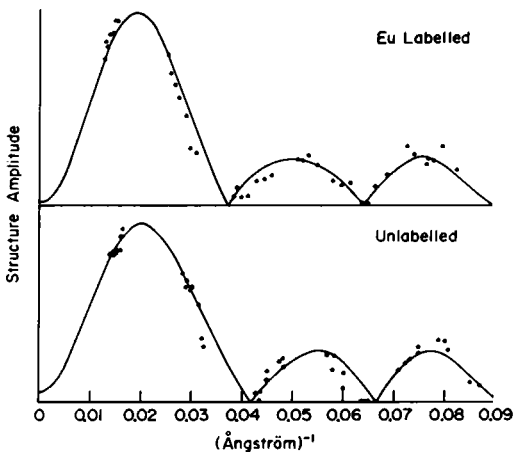

FIGURE 1 Structure amplitudes for a series of swelling experiments for unlabelled and europium-labelled PC:G_{M1} multilayers. The smooth curves were calculated using the sampling theorem.

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