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Perspective

Reevaluating Equilibrium and Kinetic Binding Parameters for Lipophilic Drugs Based on a Structural Model for Drug Interaction with Biological Membranes

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Introduction

Structure–function activity relationships of drugs that bind to certain membrane-associated receptors must take into account the local membrane bilayer environment where the binding event occurs. The partitioning of drugs in an isotropic two-phase bulk solvent system such as octanol/buffer apparently is not a good model for drug interaction with the lipid bilayer of membranes. Knowledge of these membrane-based partition coefficients then necessitates reanalysis of other physical, chemical, and functional parameters.

In this Perspective, we have reexamined the model used in the equilibrium dissociation constant (K_d) determination for certain lipid-soluble drugs based on recent experimental data describing the interaction of these drugs with the membrane bilayer. Because several lines of experimental evidence suggest that some lipophilic drugs bind to hydrophobic, intramembrane receptor sites via the membrane bilayer, the concentration of such drugs in the membrane bilayer compartment in equilibrium with the receptor needs to be considered for K_d calculations. In other words, instead of expressing the "free" and "bound" concentrations of the drug in terms of a total aqueous volume (moles of drug per liter of solution), these quantities should be expressed as a function of the membrane lipid volume (moles of drug per liter of membrane lipid). The results of this analysis indicate that K_d values calculated on the basis of an aqueous concentration of the drug are significantly different from those using the "membrane concentration" of the drug, as measured experimentally. This difference in the K_d values is related to the membrane partition coefficient of the drug.

In addition to affinity constants, drug interaction with the membrane should be considered for other pharmacological parameters such as pK's and association rate constants. These parameters are important considerations for designing new therapeutic agents that have a dominant interaction with a cell membrane and a specific component of a cell membrane.

Molecular Models for Drug Binding to Membrane Receptors

Generally, the mechanism for drug binding to a plasma membrane receptor has been considered to be analogous to that of endogenous ligands such as hormones, growth factors, neurotransmitters, etc. These agonists are generally water soluble and thought to bind to an extracellular portion of the receptor. For example, the charged acetylcholine neurotransmitter and its competitive antagonist bind to an extracellular portion of the α subunit near the opening of the ion channel.[1]

In contrast to ligand binding directly from the aqueous, extracellular environment, there is experimental support for highly lipophilic drugs to bind via the membrane bilayer.[2] For example, local anesthetics that are noncompetitive blockers (NCB) bind to the acetylcholine receptor at a site distinct from that of the agonist.[3] Photoaffinity labeling experiments suggest that the binding site for NCB

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to the acetylcholine receptor is deep in the pore of the channel, in a transmembrane region.[4,5] In addition, the activity of some of these anesthetics parallel their hydrophobicity. Electron spin resonance (ESR) studies examined the binding of reversibly charged forms of an NCB anesthetic, 2-[*N*-methyl-*N*-(2,2,6,6-tetramethyl-1-oxypiperidin-4-yl)amino]ethyl 4-(hexyloxy)benzoate (C6SL) to the receptor.[6] The charged form of the anesthetic binds only when the channel is open. However, when the channel is closed, only the uncharged form of the anesthetic (as controlled by pH) can bind to the high affinity receptor, presumably through the lipid phase. ESR experiments indicate the uncharged compound is associated with the membrane hydrocarbon core and thereby binds to the receptor protein following diffusion through the membrane.[6]

Anesthetic drug access to the acetylcholine receptor via the membrane bilayer is also supported by patch clamp studies. Despite a high-resistance membrane patch seal enclosing acetylcholine receptors, microperfusion of the anesthetic isoflurance into the medium outside of the patch resulted in altering channel activity within the patch.[7] The presence of the high-resistance seal suggested that the compound gained access to the receptor through the lipid phase.

Evidence for an intrabilayer receptor site that must be accessed by diffusion through the lipid phase has also been implicated for the β-adrenergic receptor. The human genes for both the α_2 and β_2 adrenergic receptors have been cloned and expressed in *Xenopus* oocytes. The receptors are homologous and contain seven hydrophobic domains that have been modeled as seven transmembrane spanning segments.[8] Deletion mutations have indicated that the seventh membrane spanning domain is necessary for ligand binding.[8] These mutations give experimental support to a transmembrane, intrabilayer receptor site. Although certain β-adrenergic antagonists are formally charged, as in the case of propranolol, small angle neutron diffraction experiments have observed the drug's time-averaged location to be in the hydrocarbon core, near the glycerol backbone, of biological membranes[9] while the partition coefficient of propranolol into biological membrane was relatively high, $K_p > 10^3$.[10]

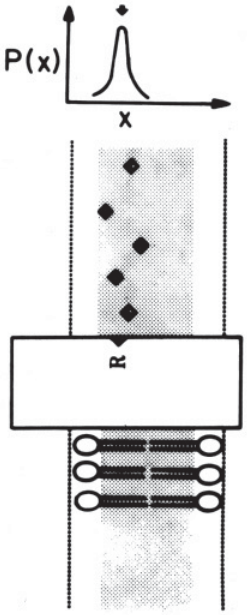

Figure 1. This figure illustrates a hypothetical transmembrane ion channel with a hydrophobic, intrabilayer receptor site labeled "R". Evidence for such a hydrophobic site is based on the DHP receptor sequence analysis (Tanabe et al., 1987) and photoaffinity labeling (Takahashi et al., 1987). Drugs that bind to this receptor site are indicated by oriented diamonds with an intrabilayer distribution profile characterized by a Gaussian curve on the right. The center of the Gaussian curve, marked by an arrow and representing the location along the bilayer normal axis of highest drug concentration, is at a depth in the membrane coincident with the drug's putative receptor site.

Finally, a "membrane bilayer pathway" has been described for the binding of lipophilic 1,4-dihydropyridine (DHP) Ca^{2+} channel blockers to voltage-dependent Ca^{2+} channels in cardiac and smooth muscle sarcolemma. This would occur in a two-step process.[11] First, the drug molecule must partition to a well-defined, energetically favorable location, orientation, and conformation in the membrane bilayer before laterally diffusing to an intrabilayer receptor binding site (Figure 1).

The primary structure of the DHP receptor subunit from rabbit skeletal muscle has been deduced from its DNA sequences. The polypeptide is structurally similar to the voltage-dependent sodium channel with four units of homology that comprise six putative transmembrane α-helices that may serve as the channel for Ca^{2+}.[12,13] In light of the high homology of the hydrophobic domains of Ca^{2+} channels with Na^+ channels, it is interesting that DHPs have been shown to bind with high affinity and stereoselectivity to the cardiac sarcolemmal sodium channel.[14] These data suggest that the specific receptor

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site for the DHPs common to both the Ca^{2+} and Na^{+} channel is a hydrophobic, transmembrane domain. Moreover, the DHP receptor subunit can be heavily labeled by a hydrophobic photoaffinity probe, indicating that the protein consists of multiple transmembrane helices.[15]

The probability that DHPs interact with the bulk lipid phase in the cardiac sarcolemma is high in light of high partition coefficients measured for several DHPs ($K_p > 10^3$, refs 10, 16–19) and the very low receptor density (approximately one receptor site per square micron in the cardiac sarcolemmal membrane; ref 20). Diffusion-limited rates calculated for a membrane pathway are approximately 3 orders of magnitude greater than those for an aqueous approach in which the drug reaches the receptor by diffusion through the bulk solvent.[11] The two-dimensional component of this process, lateral diffusion through the bilayer, has a significant rate advantage if the ligand has the appropriate location and orientation for binding to the receptor site.[21]

Experimental support for the first step of this pathway, namely DHP partitioning to a discrete, time-averaged location in the membrane bilayer, has been shown by using small-angle X-ray and neutron diffraction with several representative DHPs.[10,18,19] The second step of the membrane bilayer pathway, namely DHP lateral diffusion through the membrane, was measured by using florescence redistribution after photobleaching (FRAP). With use of an active rhodamine labeled DHP analogue, the microscopic rate of drug lateral diffusion was measured in canine cardiac sarcolemmal lipid multilayers over a wide range of relative humidities.[22,23] At the highest relative humidity, the rate of lateral diffusion for the DHP was identical with that measured for phospholipid analogs (3.8×10^{-8} cm^2/s). These rapid rates of diffusion suggest that the overall binding rate by a membrane bilayer pathway is generally not rate-limited by the drug's diffusion through the membrane.[11]

Recently, Boer and co-workers[17] in the laboratory of H. Glossman have also observed high membrane partition coefficients for DHP analogues. However, their interpretation of the relationship of these findings to the "true" K_d for DHP binding to Ca^{2+} channels did not consider the possibility of the membrane bilayer pathway as a model for DHP receptor binding. They view the high partitioning into the membrane as effecting a depletion of the active drug available in the surrounding medium for binding to an exposed receptor site by an aqueous pathway. Thus, they proposed that the true K_d was inversely related to the DHP's partition coefficient. By contrast, we propose, from a variety of studies including our own, that the relevant concentration of drug in equilibrium with the DHP receptor site is within the membrane bilayer compartment, and that there is a direct relationship between the true K_d and the DHP partition coefficient.

Table I. 1,4-Dihydropyridine Partition Coefficients into Biological Membranes and Octanol/Buffer[a]

drug	biological membranes[b] (sarcoplasmic reticulum)	octanol/buffer
Bay P 8857	125 000	40
iodipine	26 000	
amlodipine	19 000	30
nisoldipine	13 000	40
Bay K 8644	11 000	290
nimodipine	6 300	730
nifedipine	3 000	

[a] Some of the data in this table were reproduced from ref 10, 16, 18, and 19. [b] Similar values were obtained with cardiac sarcolemmal lipid extracts, indicating a primary interaction of the drug with the membrane bilayer component of these biological membranes.

Table II. Drug Partition Coefficients into Biological Membranes and Octanol/Buffer[a]

drug	biological membranes (sarcoplasmic reticulum)	octanol/buffer
amiodarone	921 000	350
beta X-61	12 500	120
beta X-67	3 200	250
propranolol	1 200	18
beta X-57	350	3
cimetidine	300	1
timolol	16	0.7

[a] Some of the data in this table were reproduced from ref 10, 16, 18, and 19.

Drug Partition Coefficients into Biological Membranes Differ Dramatically from Those Measured in Octanol/Buffer Systems

Data in Table I of drug partition coefficients highlight the fact that drug interactions with both model and biological membranes are complex and cannot be mimicked by isotropic model systems, e.g. octanol/buffer. The charged DHP Ca^{2+} channel antagonist amlodipine is a case in point. The partition coefficient measured in octanol/buffer, $K_{P[iso]}$, for amlodipine was nearly 1 order of magnitude lower than that of the uncharged DHP nimodipine. By contrast, its partition coefficient K_P in a biological membrane, $K_{P[mem]}$, is over 3-fold higher than that of nimodipine. The differences in drug partitioning into octanol/buffer versus membranes were also observed for a wide variety of drugs including antiarrhythmic agents, H_2 antagonists, and β-adrenergic blockers (Table II).

Once it has been recognized that the bilayer environment is important to drug/lipid interactions and that drugs

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assume a well defined location in membrane bilayers, it is not surprising to find that modulating the physical (e.g., thermal phase transition; ref 18) or chemical (e.g., cholesterol content; ref 24) characteristics of the membrane substantially affects the DHP $K_{P[mem]}$. These changes in the composition of native plasma membranes and their effect on drug pharmacodynamics have clinical relevance when considering the membrane compositional changes, especially in the cholesterol content, associated with aging,[25-27] chronic cigarette smoking,[28] experimental diabetes,[29] and hypercholesterolemia.[30,31] In our current studies, we have shown that an increase in membrane cholesterol from a 0:1 cholesterol:phospholipid mole ratio (C:Pl) to a 0.6:1 C:Pl mole ratio resulted in a 11-fold decrease in the $K_{P[mem]}$ of the DHP Ca^{2+} channel antagonist nimodipine (data not shown). Thus, the drug interacts with a chemically and structurally anisotropic environment in a manner that cannot be predicted from $K_{P[iso]}$.

Structural Implications of the Membrane Bilayer Model for Drug Binding: Drug-Design Concepts

The mechanism of binding for DHP calcium channel antagonists and agonists to voltage-sensitive calcium channels in the cardiac sarcolemma is a complex reaction that may involve interaction with the membrane bilayer. The hypothesis that the DHP receptor site may be within the membrane bilayer compartment is indicated from genetic studies that suggest that the DHP receptor is a hydrophobic, transmembrane protein. Thus, DHP partitioning to a discrete, energy favorable location, orientation, and conformation may be prerequisite for subsequent intrabilayer receptor recognition and binding. By reducing the degrees of freedom of the drug by limiting it to a specific region of the membrane, the phospholipid bilayer can effectively increase the efficiency of binding for low concentrations of drug to an intrabilayer receptor site.

The strong interaction of DHPs with membrane bilayers may also be helpful in understanding their side effects. DHPs may utilize a "membrane bilayer pathway" in their reactions with voltage-sensitive calcium channels in other tissues in a manner analogous to that described for the heart. For example, the cardiac drug Bay K 8644's various negative psychopharmacologic effects may result from

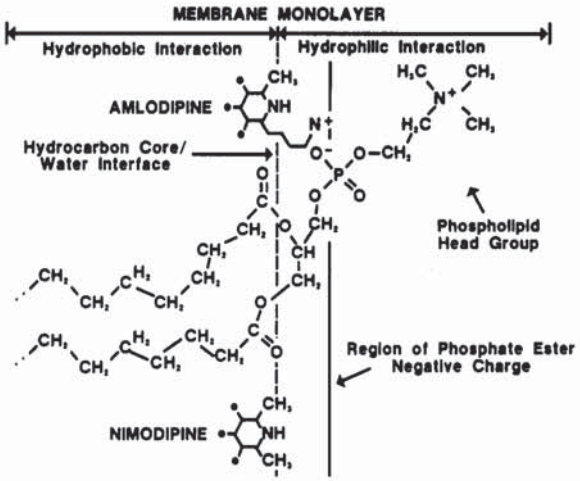

Figure 2. This figure summarizes amlodipine's interaction with the membrane bilayer in light of its determined center-of-mass location and crystal structure. The drug molecules are positioned next to a phospholipid molecule to indicate the potential chemical interactions between the molecules in this two-dimensional representation. Amlodipine's location near the hydrocarbon core/water interface can facilitate both a hydrophobic interaction with the phospholipid acyl chains and an ionic interaction between the protonated amino function of the drug and the charged anionic oxygen of the phosphate headgroup. The dihydropyridine ring of amlodipine was superimposed on that of nimodipine (using structures obtained from crystallographic analysis) at the membrane location experimentally determined by neutron diffraction for nimodipine. The nimodipine structure and location is consistent with only hydrophobic interactions with the phospholipid acyl chains and not an electrostatic interaction with the phospholipid headgroup as in the case of amlodipine. (Reprinted with permission from *Mol. Pharmacol.* 1989, *36*, 634–640.)

binding to DHP sites in the central nervious system.[32]

These data demonstrate that drug interactions with the native membrane bilayer are complex. Clearly, the chemical and crystal structure of the drug alone does not provide sufficient information with which to predict certain drug–membrane interactions. Moreover, traditional scientific methods to assess the "lipophilicity" of drugs by measuring partition coefficients into nonpolar alkane solutions such as octanol/buffer appeared to be inadequate for certain drugs on the basis of the results of this study. The anisotropic bilayer structure, in contrast to a bulk phase solvent such as octanol with invariant properties throughout, has very different physical and chemical characteristics as a function of distance across the bilayer normal axis that will affect drug–lipid interaction. Drug partitioning and location in the bilayer appeared to exploit these differences to achieve an energetically favorable location, orientation, and conformation.

Small-angle X-ray diffraction experiments also showed the "specificity" of nonspecific drug interactions for DHPs with the membrane bilayer. While in octanol, the DHP was randomly dispersed throughout the solution, in a membrane bilayer the DHP occupies a discrete, time-averaged location near the hydrocarbon core/water interface. This location can facilitate both hydrophobic and ionic interactions of amlodipine with neighboring phospholipid molecules (see Figure 2). These structural results were

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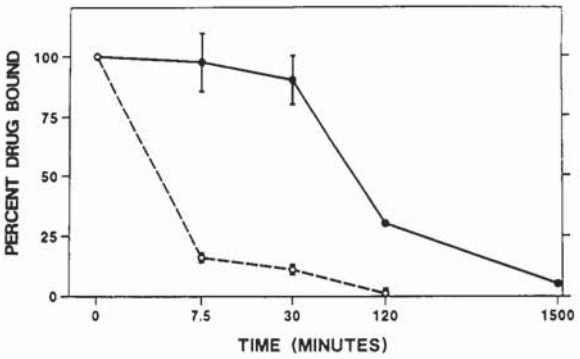

Figure 3. Nonspecific dissociation of 1×10^{-9} M [^{3}H]amlodipine (solid circles) and 1×10^{-9} M [^{3}H]nimodipine (open circles) from light sarcoplasmic reticulum membrane vesicles. This figure shows the percentage of drug nonspecifically associated with the membranes as a function of time. (Reprinted with permission from *Mol. Pharmacol.* **1989**, *36*, 634–640.)

fundamental to our understanding of amlodipine's unusually high partition coefficient value into membranes versus octanol (Table I). As expected, amlodipine's charge resulted in a relatively low partitioning into octanol ($K_{P[oct]}$ = 30) when compared with the uncharged DHP, nimodipine ($K_{P[oct]}$ = 260). Amlodipine's high membrane partition coefficient ($K_{P[mem]}$ = 19000), which exceeds by 4-fold the value obtained for nimodipine ($K_{P[mem]}$ = 5000), can be explained by both its hydrophobic interactions with the membrane hydrocarbon core in addition to its very favorable ionic bonding with the anionic oxygen of the phospholipid headgroups (Figure 2). These membrane interactions were deduced from the X-ray diffraction structure studies.[19]

In addition, amlodipine's membrane interactions may be a clue to understanding its novel pharcodynamic and pharmacokinetic profile, including a slow onset and long duraction of activity in vitro and in vivo relative to uncharged drugs of this class.[33] For example, amlodipine remained bound to LSR membranes 1 order of magnitude longer than the uncharged DHP, nimodipine (Figure 3). The location of amlodipine at the hydrocarbon core/water interface of the membrane is similar to that observed by X-ray and neutron diffraction for the uncharged DHPs Bay K 8644[18] and nimodipine,[10] suggesting a common, energetically favorable hydrophobic interaction with the fatty acyl chain region near the glycerol backbone. In addition, however, amlodipine may have an ionic interaction between its protonated amino function and the charged anionic oxygen of the phosphate headgroup. Specifically, if one superimposes the DHP ring of amlodipine with that of nimodipine (using structures obtained from crystallographic analysis) at the membrane location experimentally determined by neutron diffraction for nimodipine, the charged amino function of amlodipine can be placed in a region for effective ionic interaction with the anionic oxygen atom of the phosphate ester (Figure 2). This additional charge–charge interaction for amlodipine may be the basis for its longer, nonspecific association with the membrane and its unusual pharmacodynamics and pharmacokinetics described above. However, using crystal structure data to predict the drug structure in a membrane may not always be valid since the crystal and energy-minimized membrane bilayer structures of amlodipine may differ, as will be discussed in the next section. Further structure studies would be necessary to confirm amlodipine's orientation and conformation in the membrane for comparison to other uncharged DHPs.

Nicardipine is also a positively charged DHP with a pK_a (7.0) lower than that of amlodipine. Although at physiological pH approximately 30% of the nicardipine molecules are charged, this compound has a pharmacokinetic half-life similar to that of uncharged DHPs. The location of the protonated amino group of nicardipine is at the C_3 position of the dihydropyridine ring, adjacent to the 4-phenyl substituent. If the DHP ring of nicardipine is at the same membrane location as that of nimodipine, the charged amino group may not be able to interact electrostatically with the charged headgroup of the membrane bilayer, even if fully extended. Further, the presence of a phenyl group adjacent to the charged tertiary amine of nicardipine would result in an energetically unfavorable interaction in the hydrophilic environment near the headgroup. Thus, despite its formal charge, nicardipine may not demonstrate the additional electrostatic interactions proposed for amlodipine. This would result in a shorter residence time in the membrane and an observed duration of activity similar to that of uncharged DHPs.

Drug Structure in a Crystal versus a Membrane

Intuitively, the substantial differences in the drug's microenvironment in a crystal matrix versus the membrane bilayer would be expected to affect its molecular conformation substantially. To test this hypothesis, small-angle X-ray diffraction was used to identify the time-averaged location of the antiarrhythmic agent, amiodarone, in a synthetic lipid bilayer as shown in Figure 4.[34] The location in the membrane was then used to assign an appropriate dielectric environment in which the determined crystallographic drug conformation could be energy minimized via the molecular mechanics program MMP2.[35] The drug was located ~6 Å from the center (terminal methyl region) of the lipid bilayer (Figure 4). Thus, a dielectric constant of κ = 2, approximating that of the bilayer hydrocarbon core region was used to calculate a minimum-energy structure for membrane-bound amiodarone. The resulting calculated structure was significantly different when compared with the crystal structure of amiodarone. These calculations did not take into consideration specific steric interactions of the lipid acyl chains on the conformation of this lipophilic drug. Nevertheless, the results of this work suggest that the biologically active conformation of a drug that interacts with an intrabilayer receptor site, for example, may be quite distinct from its crystal structure conformation.

A Membrane Bilayer Pathway Affects Assumptions for K_d Calculations: Rationale for Recalculating "Free" and "Bound" Concentration Terms

To calculate the equilibrium dissociation constant for a given drug and receptor, the amount of drug bound

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DOCKET ALARM

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Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

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Sync your system to PACER to automate legal marketing.