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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 p$K$'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 $\alpha$ 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 $\beta$-adrenergic receptor. The human genes for both the $\alpha_2$ and $\beta_2$ 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 $\beta$-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]

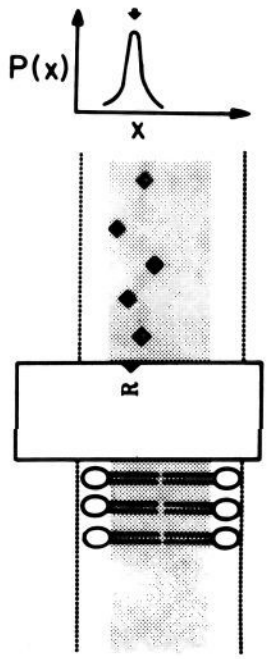


**Figure 1.** This figure illustrates a hypothetical transmembrane ion channel with a hydrophobic, intrabilayer receptor site labeled "R". Evidence for such a hydrophobic site is based on the DHP receptor sequence analysis (Tanabe et al., 1987) and photoaffinity labeling (Takahashi et al., 1987). Drugs 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 $\alpha$-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 ($3.8 \times 10^{-8}$ 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 $\beta$-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

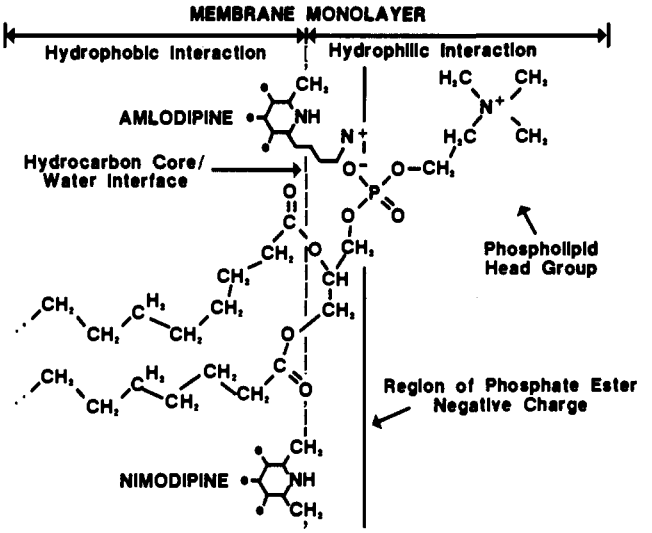


Figure 2. This figure summarizes amlodipine's interaction with the membrane bilayer in light of its determined center-of-mass location and crystal structure. The drug molecules are positioned next to a phospholipid molecule to indicate the potential chemical interactions between the molecules in this 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 nervous 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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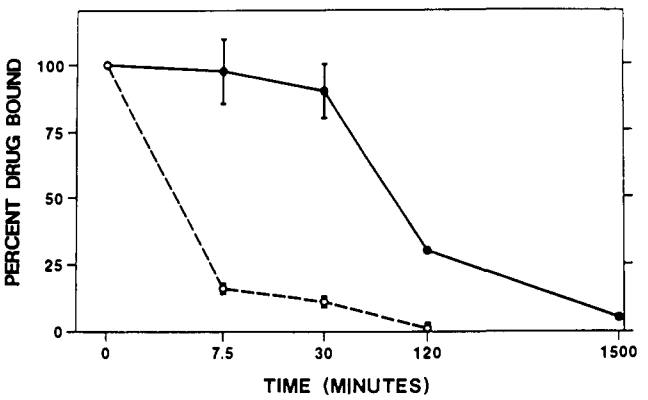


**Figure 3.** Nonspecific dissociation of $1 \times 10^{-9}$ M [$^3$H]amlodipine (solid circles) and $1 \times 10^{-9}$ M [$^3$H]nimodipine (open circles) from light sarcoplasmic reticulum membrane vesicles. This figure shows the percentage of drug nonspecifically associated with the membranes as a function 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 p$K_a$ (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 $\kappa$ = 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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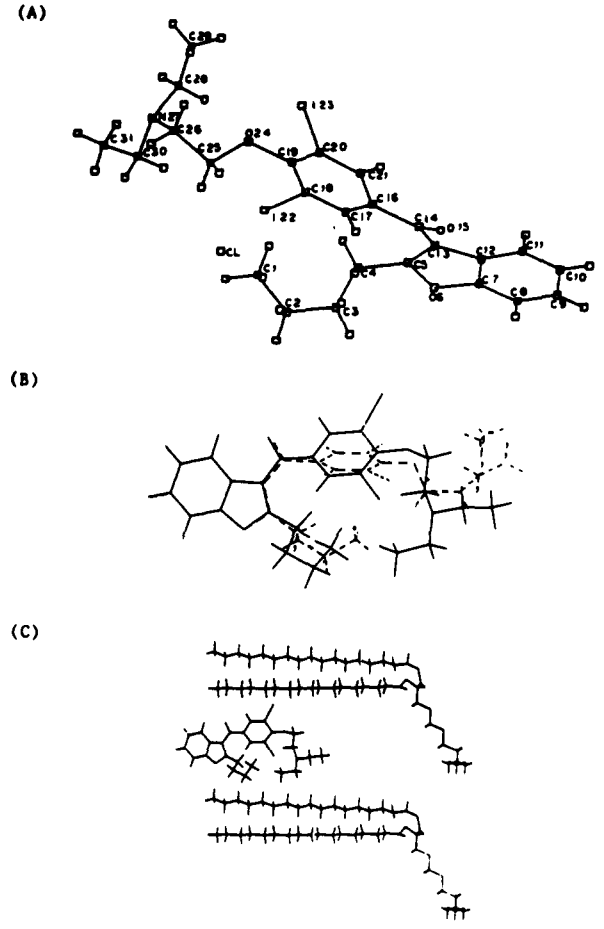

Figure 4. (A) The crystal structure of Class III antiarrhythmic agent, amiodarone. Electron-dense iodine atoms that are part of the covalent structure of this drug are strong scattering sources for the incident X-ray radiation. (B) The minimum-energy structure of amiodarone (solid lines) calculated in a low dielectric medium with the program MMP2 is superimposed on the crystal structure of the drug (dashed lines). (C) The location of amiodarone in a lipid monolayer based on the results of X-ray diffraction. The center of mass of the iodine atoms were observed to be 6 Å from the membrane bilayer center. (Reprinted with permission from *Biophys. J.* 1988, *54*, 535–543.)

(concentration of drug in the mixture present as a drug–receptor complex) and free (concentration of drug that is not associated with receptor) must be accurately determined at equilibrium. In conventional Scatchard analysis, both of these parameters are expressed as "overall" molar concentrations (i.e. the moles of drug per liter of reaction mixture) and by plotting data as bound/free versus free, an apparent equilibrium dissociation constant ($K_d$) can be obtained.

This analysis may apply to a variety of lipophilic drug molecules that bind to an intrabilayer receptor site. For this study, we will focus on the DHP $Ca^{2+}$ channel modulators. Previous DHP $K_{P[mem]}$ measurements by our laboratory[10,18,19] using cardiac membranes and recent measurements by Boer et al.[17] have prompted us to redefine the drug concentrations used for Scatchard analysis. The $K_{P[mem]}$ for these drugs were greater than $10^3$ (see Table I), indicating that the drug was present in the membrane volume at concentrations substantially higher than in the extra-membrane, aqueous surroundings.

The unbound drug in the membrane was free to diffuse laterally within the membrane bilayer with a rate of 3.8 $\times 10^{-8}$ cm$^2$/s [22] and bind to its specific membrane receptor. Thus, the drug concentration in the membrane bilayer might be more appropriately used as the "free" component for specific binding calculations.

## Reevaluation of DHP Equilibrium Constants Based on Drug Concentration in the Membrane

Consider the bimolecular ligand–receptor reaction: R + D $\rightleftharpoons$ RD. The overall association constant (including the entire, aqueous volume), $K_a'$, is

$$K_a' = C_{RD}/C_R C_D \quad (1)$$

where $C_R$, $C_D$, and $C_{RD}$ are the molar concentrations of free receptor sites, free ligand, and receptor bound ligand, respectively. If $C_R^0$ is the initial (total) concentration of receptor sites ($C_R^0 = C_R + C_{RD}$), then

$$C_{RD}/C_D = K_a' C_R^0 - K_a' C_{RD} \quad (2)$$

where $K_a'$ is the apparent association constant based on the conventional Scatchard analysis, and the concentration terms ($C_{RD}$, $C_R^0$, and $C_D$) are relative to the total volume of the solution. If, however, we consider the "local", intramembrane equilibrium we must redefine the pertinent concentrations. These may be expressed in terms of the total solution-based concentrations as

$$C_{RD}(mem) = C_{RD}(V_T/V_M)$$

$$C_R^0(mem) = C_R^0(V_T/V_M)$$

$$C_D(mem) = C_D(K_{P[mem]})$$

where $V_T$ and $V_M$ are the total volume and the membrane bilayer volume, respectively (the minor correction due to density differences of lipid versus aqueous solution was neglected in these calculations). As previously described, the DHP molecules are not randomly distributed throughout the membrane bilayer volume but have a time-averaged location near the hydrocarbon core/water interface. Thus, the actual volume of the membrane occupied by the drug is substantially less than the total membrane volume, $V_M$. Furthermore, the DHP concentration at the receptor site is dependent on the location of the receptor site in the membrane compartment relative to the drug's membrane location (see below). Rewriting eq 2:

$$\frac{C_{RD}(V_T/V_M)}{C_D K_{P[mem]}} = K_a C_R^0(V_T/V_M) - K_a C_{RD}(V_T/V_M) \quad (3)$$

which can be expressed in the form of the Scatchard equation:

$$C_{RD}/C_D = K_a K_{P[mem]} C_R^0 - K_a K_{P[mem]} C_{RD} \quad (4)$$

This shows that the experimentally measured equilibrium association, $K_a'$, is a composite of the association constant for the local, intramembrane equilibrium, $K_a$, and the partition coefficient, $K_{P[mem]}$. The local, true equilibrium constant, $K_a$, can be calculated from the overall association constant and the membrane-based partition coefficient:

$$K_a = K_a'/K_{P[mem]}$$

For example, the intrinsic affinity of nimodipine for its receptor site is over 3 orders of magnitude less than that based on an overall equilibrium constant that ignored the interaction of the drug with the membrane. Similarly, $K_d$ (defined for the intramembrane equilibrium) is related to the overall dissociation constant, $K_d'$, by the relationship $K_d = K_d' K_{P[mem]}$ (see Table III). This result is consistent with the model presented by Parry and co-workers[36] which

Table III. Calculation of Equilibrium Dissociation Constants Based on the Drug's Membrane Concentration

| drug | $K_{P[mem]}$ (from Table I) | $K_d'$, (aqueous concn) | $K_d$, M (membrane concn) |
|---|---|---|---|
| nimodipine[a] | 6300 | $1.1 \times 10^{-10}$ | $6.9 \times 10^{-7}$ |
| nifedipine[b] | 3000 | $4.1 \times 10^{-10}$ | $1.2 \times 10^{-6}$ |
| Bay K 8644[c] | 11000 | $2.4 \times 10^{-9}$ | $2.6 \times 10^{-5}$ |
| nisoldipine[b] | 13000 | $1.4 \times 10^{-10}$ | $1.8 \times 10^{-6}$ |
| amlodipine[d] | 19000 | $1.2 \times 10^{-9}$ | $2.3 \times 10^{-5}$ |
| iodipine[e] | 26000 | $3.9 \times 10^{-10}$ | $1.0 \times 10^{-5}$ |

[a] Canine cardiac muscle (ref 16). [b] Rat cardiac muscle (ref 39). [c] Rabbit cardiac muscle (ref 40). [d] Rat smooth muscle (ref 33). [e] Guinea pig skeletal muscle (ref 41).

proposed that the $K_d$ would increase with the membrane partition coefficient for a lipophilic compound interacting with an intrabilayer receptor site. In the case of their study, they considered lipophilic substrate interaction with the P-450 enzyme.

The significance of these considerations for drug design is obvious; a compound with high overall activity may result from high specific affinity for the receptor site, a high $K_{P[mem]}$, or a combination of these factors. These factors become problematic when one realizes that for DHPs whose apparent affinity is due to high $K_{P[mem]}$'s, these drugs will be present in proportionately higher membrane concentrations. On the basis of experimentally determined molar ratio of lipid to DHP receptor in CSL of $4.2 \times 10^6$ (20), there should be 10 nimodipine molecules per receptor in the membrane at a concentration of $\sim 1 \times 10^{-10}$ M corresponding to the aqueous $K_d'$. Nisoldipine, however, has a $K_d'$ comparable to that of nimodipine, but a partition coefficient approximately 2-fold higher. Thus, the intrinsic affinity of nimodipine for the site is weaker, the requisite intrabilayer concentration for a given response is higher, and the probability of deleterious side effects is greater.

It must be remembered that this discussion assumes that all drug in the membrane is available for interaction with the active site on the receptor. It has been shown that many drugs, and the DHP's in particular, are not uniformly distributed thoughout the membrane bilayer, but are restricted to specific locations along the bilayer normal axis, i.e. along the fatty acyl chain axis.[10,18,19,24] Thus, the apparent concentration could be 10-fold higher than that calculated assuming that the pertinent membrane volume is that of the entire membrane. On the other hand, although there is some data to suggest that the DHP active site is in the intrabilayer compartment,[15,37] it is possible that the site and drug will not be at the same depth in the membrane bilayer. In this case, the actual drug concentration at the site could be lower than would be predicted on the basis of $K_P$ analysis, and might even be lower than the aqueous concentration. By a more detailed analysis, one could experimentally determine $K_{P[mem]}$ and the intrabilayer distribution profiles for the drug and site (see Figure 1). The effect of such variations would be to modulate the apparent association constant, $K_a$.

In a practical sense, this analysis may provide a better explanation for some structure/activity data for DHPs and other drugs that act on membrane-associated receptors. Beyond the traditional octanol/water correlations that have been examined so extensively, a detailed study of the molecular interaction of the drug with the environment of interest might be warranted.

## Other Implications of the "Membrane Bilayer Model"

The data and arguments provided above regarding membrane solubility of DHPs extend to nearly any membrane active drug that uses the membrane as a vehicle for facilitating the interaction of that drug with its protein receptor. This model and its implications can extend to such drugs as the benzodiazepines, which apparently interact with the surface of the membrane as part of their binding to the GABA benzodiazepine receptor in synaptoneurosome membranes.[38] Thus, a variety of physical chemical properties of drugs warrant reexamination in light of drug binding by a membrane bilayer mechanism. For example, the p$K$ of an ionizable drug will be different following partitioning into the membrane hydrocarbon core where the dielectric constant is $\sim 3$ compared to the p$K$ in an aqueous environment dielectric constant of 80. Perhaps, for certain membrane active agents for which a protonated but ionizable group is forced into the bilayer interior, p$K$ values determined in solvents with (low) dielectric constants would be more appropriate.

## Significance of Determining Molecular Information for Drug Design

The combined X-ray and neutron diffraction approach provides valuable information regarding the molecular interactions of drugs with membranes. Apart from the significance in determining mechanisms for drug–membrane and drug–receptor actions, these data can be used to define the activities and selectivities of drug substances. A drug that does not penetrate to the proper depth within the membrane bilayer (drug y in Figure 5B) would not be as active as drug x, which at the proper depth within the membrane bilayer can diffuse to the receptor site and participate in a successful interaction. This feature of the model may also accommodate a selection for activity, whereby only a portion of the drug structure is the "active site", so that a hydrophobic (that portion of the drug within the hydrocarbon core) or a hydrophilic (that portion of the drug within the aqueous region of the membrane bilayer) interaction may occur. The limited amount of information to date indicates that cardiovascular agents have precise locations (i.e. depth of penetration) within the membrane bilayer, and this finding should not be ignored. The model provides another way of selecting the proper drug by its orientation within the membrane. Drug y in Figure 5C will not be active, because its orientation does not match that of the receptor site, whereas drug x in Figure 5C does. In addition, conformation may be im-

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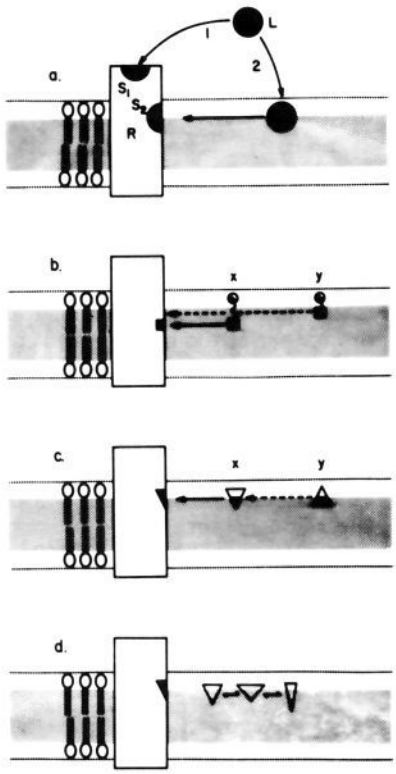


**Figure 5.** (A) A drug (L) may reach a binding site ($S_1$ or $S_2$) on a membrane-associated receptor protein (R) by direct diffusion through the aqueous surroundings or by partitioning into the lipid bilayer and then laterally diffusing to the active site. (B) The highly ordered structure of the lipid bilayer may restrict lipophilic or amphiphilic drugs to a particular depth of penetration in the bilayer; drug x would be a relative molecule, because it is positioned at the proper depth of penetration for optimal reaction with a protein–receptor site, whereas drug y would be inactive or partially reactive. (C) The orientation of these drugs relative to the active site might also be optimized by the constraints of the lipid-bilayer structure by limiting the drugs' degrees of rotational freedom; again, drug x would be active, drug y would be inactive. (D) Due to conformational equilibria not necessarily similar to those in bulk phases, a conformation suitable for binding to a receptor site may exist only as a metastable state. It is likely that when the drug enters the bilayer, its conformation changes. It is this conformation that must interact with a site on a protein with a particular, corresponding conformation.

portant as demonstrated in Figure 5D. The lipid bilayer may constrain the flexible portions of drug structures in a given conformation relative to the inflexible regions of the drug molecule, thus setting up different overall drug conformations that are dependent on the lipid bilayer structure and composition. When the drug leaves the lipid phase and first makes contact with the protein receptor site in direct contact with this lipid phase, the drug conformation as mandated by the lipid bilayer may now be optimized for binding to the protein receptor site. Finally as shown in Figure 1, since drug location is "smeared" as a distribution about some average location, the precise location of the drug in this distribution may help to explain partial reactivity of the drug. Thus, if the optimal location for the protein receptor site is at the peak of the distribution, but a given drug structure partitions to some other location, then this particular drug structure may only have partial reactivity for this receptor site. The limited amount of structure data to date also points to precise orientations and conformations of these cardiovascular agents within the membrane bilayer, and this probably should not be ignored.

These structure tools may become part of an overall integrated system composed of three parts that could allow new breakthroughs in drug design. Single-crystal studies of drug structures (obtained by both X-ray and neutron diffraction) could be combined with drug structures (again obtained by X-ray and neutron diffraction) determined directly in biological membranes. These experimentally determined structures for the drug in crystal and membrane-bound forms could be put into molecular modeling schemes. On the basis of studies of several drug substances in a particular class, similarities and/or differences of the drug structures in the crystal and membrane-bound forms, in addition to their structures when bound at a protein receptor site, will allow calculations of potential site reactivity and selectivity. Optimized drug structures calculated in this manner could then be synthesized, and both their structures and biological activities could then be determined, completing the cycle. This cyclic scheme of combining experimentally determined drug structures (crystalline versus membrane-bound form) with molecular modeling and predicting new structures should allow one to optimize therapeutic agents within a particular class of drugs, and possibly predict new drug compounds with clinical potential.

## Conclusion

The affinity of a drug for its specific receptor is a quantity that is fundamental to understanding structure/function relationships for drug design. In light of a membrane bilayer pathway for drugs targeted to an intrabilayer receptor, the basic calculations for equilibrium constants were reexamined in this paper. For example, Table I showed the substantial difference (of at least 3 orders of magnitude) in the aqueous concentration compared to that in the membrane. This drug concentration in the membrane is generally ignored in conventional equilibrium calculations, e.g. Scatchard analysis, resulting in affinity results based on the significantly lower aqueous drug concentration. Thus, while iodipine has an aqueous $K_d'$ similar to that of nifedipine, the actual concentration of iodipine (based on measured $K_{P[mem]}$ values) in the membrane will be approximately 10-fold higher than nifedipine at this aqueous $K_d$ concentration. Thus, the apparent affinity of these DHPs for their receptor based on aqueous concentrations alone can be misleading. By extrapolation, association rate constants would be similarly affected since they are dependent on the drug concentration. Off-rate constants should be unaffected unless there are still other factors in addition to the concentration of the drug that can come into play. The significant amounts of unbound drug in the membrane may result in deleterious side effects by binding to other membrane proteins. Further understanding of the interactions of DHPs and other lipophilic drugs with the membrane en route to an intrabilayer receptor site is necessary for the design of drugs with greater efficacy and reduced side effects.

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# *Articles*

## Synthesis and Resolution of (±)-7-Chloro-8-hydroxy-1-(3′-iodophenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (TISCH): A High Affinity and Selective Iodinated Ligand for CNS D1 Dopamine Receptor

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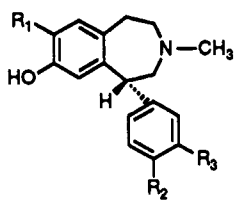
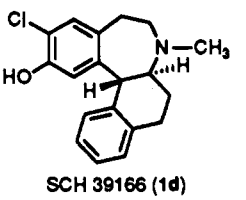
The synthesis and resolution of (±)-7-chloro-8-hydroxy-1-(3′-iodophenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine, (±)-TISCH (8) has been achieved by resolution of intermediate 4, the *O*-methoxyl, 3′-bromo derivative, as the diastereomeric camphor sulfonate salt. The final products, *R*-(+)-8 and *S*-(−)-8, were prepared by treatment of *R*-(+)- or *S*-(−)-7, the 3′-tributyltin intermediates, with iodine in chloroform, followed by O-demethylation. By using HPLC with a chiral column, the optical purity (>99%) of the intermediates and the final compounds was determined. Radioiodination was achieved by an iodo-destannylation reaction with sodium [$^{125}$I]iodide and hydrogen peroxide. As expected, the *R*-(+)-[$^{125}$I]-8 (the active isomer) displayed high affinity and selectivity to the CNS D-1 receptor in rat striatum tissue preparation ($K_d$ = 0.205 nM). The rank order of potency was as follows: SCH-23390 (1a) > (±)-8 > (+)-butaclamol > spiperone, WB4101 > dopamine, 5-HT. After an iv injection, the *R*-(+)-[$^{125}$I]-8 penetrated the blood–brain barrier with ease and displayed specific regional distribution corresponding to the D-1 receptor density, while the *S*-(−)-[$^{125}$I]-8 showed no specific uptake. The data suggest that the ligand may be useful as a pharmacological tool for characterizing the D-1 dopamine receptor. When labeled with I-123, this ligand is a potential agent for in vivo imaging of CNS D-1 dopamine receptor.

Dopamine receptors have been classified into two subtypes on the basis of the ability of agonists and antagonists to discriminate between two different D-1 or D-2 dopamine receptors.[1-5] These two subtypes exert synergistic effects on the activity of CNS dopaminergic neurons in rats.[6,7] Reports have suggested that D-1 and D-2 agonists invariably show opposite biochemical effects: D-1 agonists stimulate adenyl cyclase activity, while D-2 agonists inhibit this enzyme's activity.[8,9] It has been well documented that SCH-23390 (1a) (Chart I) and the related benzazepine derivatives are highly selective central D-1 dopamine antagonists.[10-16] The corresponding bromo (SKF-83566, 1b),[17] and iodo (SCH-23982, SKF-103108A or IBZP, 1c)[18-22] compounds have also been shown to have a high specificity for central D-1 dopamine receptors. A high-affinity conformationally restricted analogue, SCH-39166, (1d), ($K_i$ = 1.9 nM, against binding of [$^3$H]-1a),[23-25] and a photoaffinity ligand, *R*-(+)-IMAB (1e), were also reported ($K_d$ = 0.28 nM).[26] In conjunction with PET, [$^{11}$C]-1a showed the highest concentration in the basal ganglia area of human brain,[27,28] and two iodinated analogues, [$^{123}$I]-1c[22,31] and (±)-[$^{123}$I]FISCH (1f),[31-33] were prepared. These iodinated analogues are potentially useful for in vivo imaging with single-photon-emission-computed tomography (SPECT). An imaging study in a monkey using [$^{123}$I]-1c specifically for mapping of the CNS D-1 dopamine receptor demonstrated that the agent localized in the basal ganglia area where D-1 receptor concentration is high.[34] However, the target to nontarget ratio in brain of this

**Chart I**




SCH 39166 (1d)

| *R* -isomer compd | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| SCH-23390 (1a) | Cl | H | H |
| SKF-83566 (1b) | Br | H | H |
| SCH-23982 (1c) | I | H | H |
| IMAB (1e) | Cl | $N_3$ | I |
| FISCH (1f) | Cl | I | H |

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