Chromatographic Indexes on Immobilized Artificial Membranes for Local Anesthetics: Relationships with Activity Data on Closed Sodium Channels

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Purpose. To elucidate the effectiveness of the different parameters for the prediction of biological activity, the *n*-octanol/buffer partition coefficients and theoretical calculated lipophilicity parameters of thirteen local anesthetic drugs (LAs), including two β-blockers, were compared to the affinity values for phospholipids, calculated by a recent technique.

Methods. Interactions with phospholipids were measured by high performance liquid chromatography on a stationary phase made up of phospholipids, the so-called "Immobilized Artificial Membrane" (IAM). Reference lipophilicity parameters were measured by shake-flask method between *n*-octanol and buffer phases.

Results. Interactions with phospholipids were predicted from log P for all compounds except tocainide, which also showed additive polar extra-interactions. Moreover, when the retention on Immobilized Artificial Membrane (IAM) phase was mainly lipophilicity-based, a unique scale included the correlation between log k_w^{IAM} and log P values, for both LAs (bases) and the structurally unrelated (nonionizable and acidic) compounds previously studied. IAM interaction values for LAs were predictive of the partition measures on liposome membranes already reported in literature. The half-blocking doses for closed sodium channel, corrected for ionization at pH 7.4, were successfully correlated with the respective IAM values for eleven compounds while procaine and tetracaine, which are ester-linked compounds and have a p-amino group as well, gave more potent results than predicted by phospholipid interactions.

Conclusions. The IAM chromatographic parameters were much more effective than reference lipophilicity values in describing partition on model membranes and in predicting pharmacological potency on closed sodium channels.

KEY WORDS: local anesthetics; HPLC; immobilized artificial membrane (IAM); sodium channels.

INTRODUCTION

Structure-activity relationships of drugs that bind to some membrane associated receptors must take into account the local membrane bilayer environment where the binding events occur. For this reason much effort has been devoted to recognizing reliable and convenient *in vitro* systems to obtain physico-chemical parameters capable of predicting the distribution of drugs between polar and non-polar compartments in the organism. The driving force determining the partitioning of drugs into biological membranes is thought to be molecular lipophilicity. It is generally expressed as the logarithm of partition coefficient between *n*-octanol and buffer (log P) (1). However, in contrast to a bulk phase solvent such as octanol with invariant properties throughout, the anisotropic bilayer structure has very different physico-chemical properties due to the distances across the bilayer normal axis that will affect drug-lipid interactions.

Alternative lipophilic parameters include data from theoretical calculation, CLOGP, and chromatographic capacity factors on hydrocarbon stationary phase by High Performance Liquid Chromatography (RP-HPLC) (2–6). Although the CLOGP method is widely recognized as the industry standard, it has important limitations because the effects of intramolecular interactions (e.g., hydrogen bonds) are often improperly calculated. On the other hand, the HPLC method offers a number of advantages: sample purification is unnecessary, the partition coefficents of a mixture can be measured simultaneously, and only a minimum amount of compound is necessary for the measurements. Unfortunately, due to its high lipophilicity, the partitioning phase is a poor simulation of membrane structures and cannot model the polar interactions of drugs with phospholipids.

Therefore all these methods for assessing lipophilicity often appear to be inadequate to describe drug interactions with biomembranes. In particular, the interactions of ionized molecules with biomembranes can be complex, including both polar and lipophilic interactions (7,8).

To avoid these problems, alternative experimental methods have been developed. The use of liposome suspensions to determine the degree of interaction between solutes and biomembranes has been shown to be effective in evaluating polar interactions, but its application is limited by the fact that the technique is experimentally laborious (9).

Recently, a new HPLC stationary phase material composed by monolayers of lecithin, the so-called Immobilized Artificial Membrane (IAM) has become available (10). For ionizing compounds, our studies on this new stationary phase have revealed that IAM measures give different information when compared to conventional lipophilic parameters (11–12). Interaction studies on neutral, acidic, and basic molecules with phospholipids by IAM have demonstrated that for nonionizable compounds the log P scale effectively paralleled the scale of IAM chromatographic capacity factors extrapolated to 100% aqueous phase (log k_w^{IAM}), whereas the IAM interaction scale for ionizing compounds was distinctive from the one obtained by log P values. In fact, in some cases phospholipids seemed able to counteract the influence of electrically charged functions of analytes on lipophilic interactions. Moreover, basic molecules, such as amlodipine and nicardipine, showed attractive polar extra-interactions with phospholipids that were also observed for piroxicam, an amphoteric molecule. In particular, the presence of a basic function on analytes appeared as an essential prerequisite for the occurrence of additive polar extra-interactions. However, the role played by different pKa and kinds of aminic function (primary, secondary or tertiary) as well as by structural motifs (e.g., distance of ionized moiety from lipophilic core) is still unclear. In this study we considered a set of thirteen local anesthetic (LAs) structures amide- and ester-linked (including

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two ether-linked β-adrenergic blockers) made up of primary, secondary and tertiary amines. The inclusion of β-blockers in the set of the anesthetics was made with the purpose of investigating also sodium channel blocking agents having ether-linked structures (13). We compared the conventional lipophilic indexes, log P, with the chromatographic parameters obtained by IAM system. Our aim was to investigate the factors governing the interactions between phospholipids and basic molecules possessing different structural characteristics. Finally, it is assumed that a major source of action mechanisms for LAs is the blocking of sodium channels located in the membrane phospholipid bilayer. Evidence indicates that LAs access the receptor site via the lipid phase of membrane bilayer (14). Since an important determinant of potency for the amide-linked series was found to be its lipid solubility, expressed by CLOGP parameter (15), we decided to verify the role played by the interaction of LAs with phospholipids in determining their pharmacological effects.

EXPERIMENTAL SECTION

Materials

All local anesthetics were generously supplied by Astra Farmaceutici SpA (Milano, Italy) with the exception of mepivacaine, procaine, lidocaine, bupivacaine, alprenolol and propranolol that were from a commercial source. All chemicals were of analytical grade and used without further purification.

Chromatographic System

A Model 600E liquid chromatograph (Waters-Millipore, Milford, MA) equipped with a model 7125 Rheodyne injection valve (fitted with a 20 μl loop) and a model 486 UV detector (Waters) set at 254 nm was used. The stainless-steel column was an IAM PC. MG (4.6 × 150 mm; Regis Chemical Company, Morton Grove, IL). The chromatograms were recorded by a model 746 Data Module (Millipore).

Chromatographic Conditions

The eluents were mixtures of acetonitrile and 0.10 M phosphate buffer saline (PBS) at pH 7.0 in different percentages; the flow rate was 1.0 ml/min. The aqueous portion of eluents was filtered by membrane filters (type HA, Millipore). The eluent mixtures were obtained directly from the chromatographic apparatus by mixing, at low pressure, the organic modifier and the aqueous phase that had been deaerated previously with bubbling helium. The chromatography was carried out at room temperature. LAs were dissolved in methanol ($\sim 10^{-3}$ M), and 20 μl samples were injected in the chromatograph. Chromatographic retention data are expressed by the logarithm of capacity factor, log k′, defined as $\log k' = \log [(t_r - t_0)/t_0]$, where t_r and t_0 are the retention time of the drug and a nonretained compound (methanol), respectively.

Lipophilicity Parameters from *n*-Octanol/Water System

Data were determined according to the "shake-flask" procedure (1). *n*-Octanol was used as lipophilic phase whereas the aqueous portion was a buffer at pH 12.5 (2.85 g of KCl and 0.53 g of NaOH in 1 L of water). Quantitation after partition was performed by HPLC method in order to detect the possible occurrence of hydrolisis products of the compounds.

The theoretical CLOGP values were from the literature (16).

All reported values of log k′ and log P are the averages of at least three measurements; the 95% confidence interval associated with each value was never greater than 0.04.

Statistical Analysis

For linear regression analysis, a commercially available statistical package was used on a personal computer. Requirements of significant regression analysis were complied-with (17).

RESULTS AND DISCUSSION

Figure 1 illustrates the structures of anesthetics taken into consideration, including two β-blockers (propranolol and alprenolol). Table 1 summarizes the log P values measured by the shake-flask method using *n*-octanol/buffer at pH 12.5 as partition system. At a pH value of more than two units away from pKa (pKa + 2 for bases) an ionizable compound can be assumed to exist in its total nonionized form. As seen in Table 1, the pKa values of compounds span the range of 7.4–9.6, so the lipophilicity values determined at pH 12.5 are relative to the neutral forms (log P values).

In some cases the values of lipophilicity measured (log P) and calculated (CLOGP) appreciably differed from each other. They were correlated by the following equation:

$$\log P = 0.822\ (\pm 0.112)\ \text{CLOGP} + 0.735\ (\pm 0.277) \quad (1)$$

$$n = 11 \qquad r = 0.926 \qquad s = 0.364$$

In this and in the following equations, *n* denotes the number of molecules considered in the derivation of the regression equation, *r* is the correlation coefficient, and *s* is the standard error of the estimate. Numbers in parentheses account for the standard error of the regression coefficients.

The difficult theoretical calculation of lipophilicity for compounds bearing an aminic function can account for the relatively poor correlation found between the experimental lipophilicity values and the ones calculated. In fact, the partition behavior of these molecules does not depend only on the total intrinsic lipophilicity of N-substituents but also on electronic, steric and hydrogen bonding effects of the substituents (18).

Relationship Between log k_w^{IAM} and log P

In order to obtain experimental conditions as close as possible to the physiological pH and compatible with stationary phase stability, the determination of capacity factors on the IAM column (log k'_{IAM}) was performed with eluents at pH 7.0. At this pH value, all the compounds were, although to a different extent, mainly in their ionized form (see pKa in Table 1). However, some compounds (trimecaine, alprenolol, propranolol, bupivacaine, etidocaine, and tetracaine) did not elute within a reasonable time with completely aqueous mobile phase. Hence, they had to be eluted with mobile phases containing various acetonitrile fractions (φ). A linear relationship between log k'_{IAM} and φ was found for all LAs over the range of eluent composition examined (Figure 2). Table 1 reports the logarithms

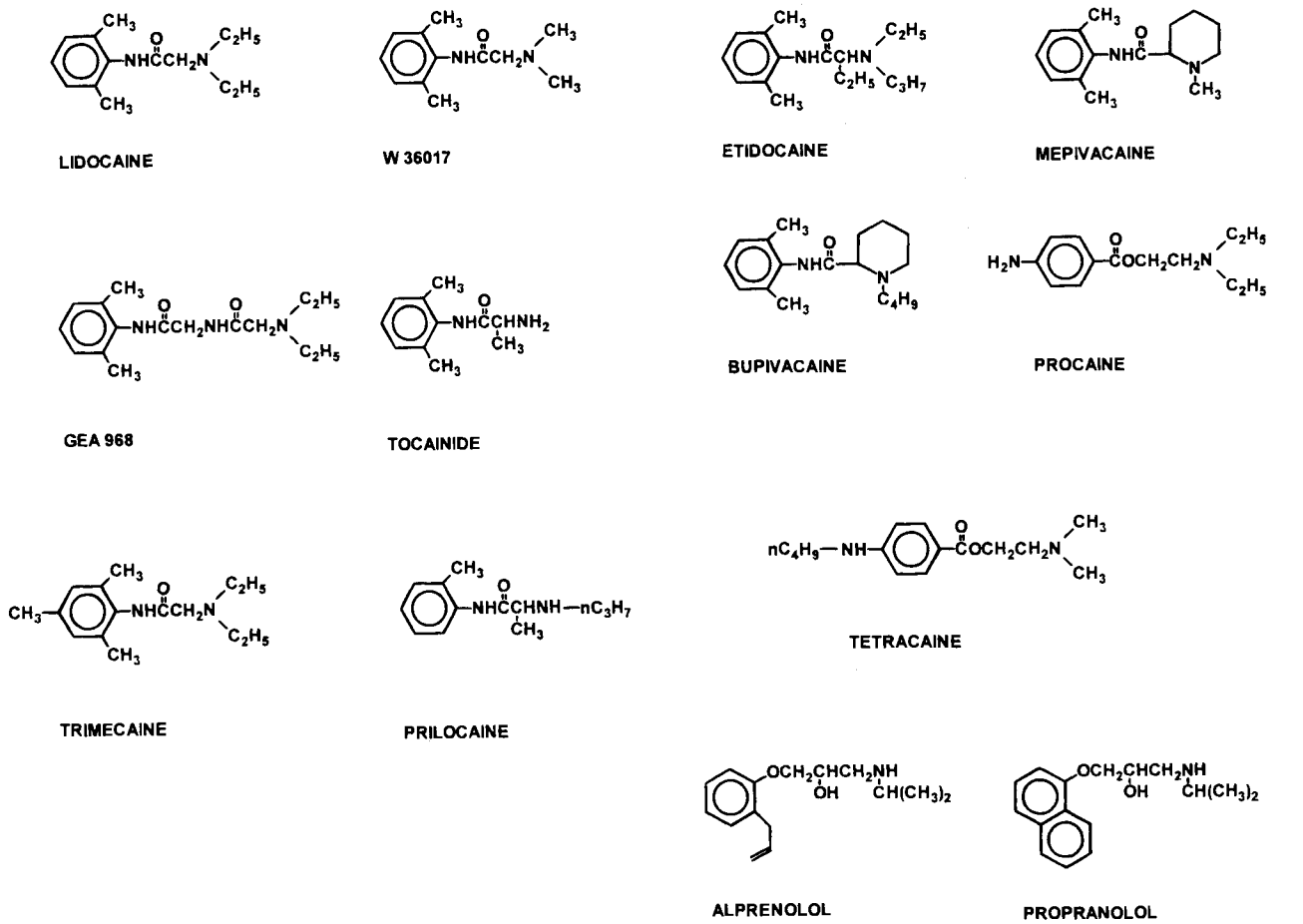

Fig. 1. Structure of investigated compounds.

Table 1. pKa Values, Lipophilicity Parameters and Capacity Factors on IAM Column, for LAs and β-blockers

Compound	pKa[a]	log k_w^{IAM}	log P	log $D_{7.0}$	CLOGP
GEA968	7.7	0.38	2.20	1.42	—
Procaine	9.0	0.39	1.95	−0.05	2.24
W36017	7.4	0.49	1.23	0.68	—
Tocainide	7.8	0.53	0.56	−0.30	−0.06
Prilocaine	7.8	0.62	2.21	1.35	1.65
Lidocaine	7.9	0.75	2.48	1.53	1.98
Mepivacaine	7.6	0.77	1.90	1.20	1.80
Trimecaine	7.4	1.21	2.73	2.18	1.86
Bupivacaine	8.1	1.45	3.40	2.27	3.38
Alprenolol	9.6	1.53	3.10	0.50	2.59
Etidocaine	7.7	1.55	3.22	2.44	3.19
Tetracaine	8.5	1.75	3.55	2.04	3.65
Propranolol	9.4	1.81	3.56	1.16	2.75

[a] values from ref. 15.

of the capacity factors extrapolated to (or measured at) 100% aqueous phase (log k_w^{IAM}).

Figure 2 shows that, as already observed for other classes of drugs (11–12), differences in the elution order occur at different percentages of the organic modifier. Hence, fictitious interaction scales only can be avoided by performing the normalization of the experimental values to 100% aqueous phase.

It is worth noting that the strongest interactions with phospholipids, which required the extrapolation procedure, were observed for the analytes having the highest log P values, regardless their pKa; e.g., propranolol showed a very high log k_w^{IAM} (1.81) although at pH 7.0 it was in its completely ionized form (pKa = 9.4). In fact, no correlation (n = 13; r = 0.568; s = 0.467) was found between log k_w^{IAM} and log $D_{7.0}$ values, i.e.

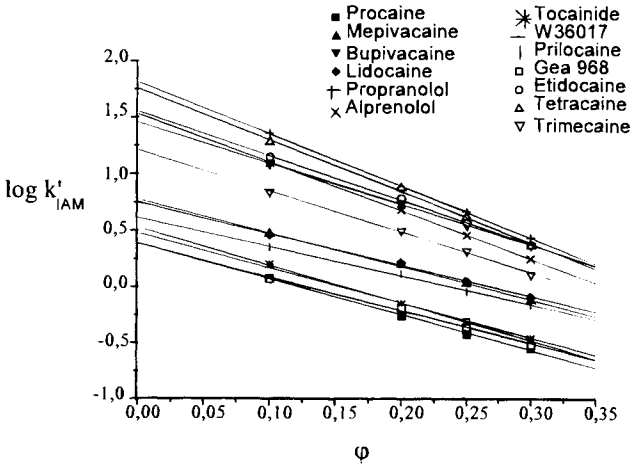

Fig. 2. Plot of logarithm of capacity factors determined on an IAM column (log k_w^{IAM}) at different fractions of acetonitrile (φ).

experimental n-octanol/water lipophilicity parameters corrected for ionization at pH 7.0. These values were calculated from the respective log P values by the following equation: log D = log P − log (1 + 10^{pKa-pH}).

A reasonable relationship was observed between log k_w^{IAM} and log P values:

$$\log k_W^{IAM} = 0.507\ (\pm 0.091) \log P - 0.234\ (\pm 0.239) \quad (2)$$

$$n = 13 \qquad r = 0.859 \qquad s = 0.291$$

This correlation indicates that the log k_w^{IAM} scale roughly parallels the one obtained by the log P values. However, the equation shows a lower correlation coefficient and a higher standard deviation than expected, indicating a quite scattered log k_w^{IAM} population. This could be a consequence of interactions, not dependent on lipophilicity, that occur between phospholipids and some of the amines considered.

Hence, to clarify the nature of the LAs/IAM interaction, we compared the log k_w^{IAM} values measured against those calculated from the respective log P (Table 2) by the following equation:

$$\log k_W^{IAM} = 0.825\ (\pm 0.040) \log P - 1.170\ (\pm 0.155) \quad (3)$$

$$n = 20 \qquad r = 0.979 \qquad s = 0.153$$

This equation, previously obtained for twenty structurally unrelated compounds (12), referred to molecules that interact with phospholipids by a uniquely partition-based mechanism. It included both neutral (hydrocarbons, 1,4-dihydropyridines) and acidic (arylacetic and arylpropionic acids) molecules with log P values spanning the range of 2.05–5.56 (benzene and lacidipine, respectively).

As can be seen in Table 2, the highest discrepancy between the log k_w^{IAM} value observed and that calculated (Δ log k_w^{IAM}) was found for tocainide, the only primary amine in the considered set of LAs. This behavior seems to indicate the occurrence of additive polar extra-interactions, and is similar to that already observed for amlodipine, another more lipophilic primary amine (log P = 3.30) (11). It is interesting to note that the discrepancies between the values observed and those expected (Δ log k_w^{IAM}) for both the primary amines, tocainide and amlodipine, were of the same order of magnitude (Δ log k_w^{IAM} were 1.04 for amlodipine and 1.24 for tocainide). Therefore, the presence of a primary aminic function on a molecule seems to support the occurrence of additive polar extra-interactions with phospholipids, regardless of the lipophilicity of the analytes.

Table 2. Observed and Calculated Log K_W^{IAM} Values for LAs

Compound	log P	log $K_w^{IAM}{}_{obs}$ [a]	log $K_w^{IAM}{}_{calc}$ [b]	Δlog K_w^{IAM}
GEA 968	2.20	0.38	0.64	−0.26
Procaine	1.95	0.39	0.44	−0.05
W36017	1.23	0.49	−0.15	0.64
Tocainide	0.56	0.53	−0.71	1.24
Prilocaine	2.21	0.62	0.65	−0.03
Lidocaine	2.48	0.75	0.88	−0.13
Mepivacaine	1.90	0.77	0.40	0.37
Trimecaine	2.73	1.21	1.08	0.13
Bupivacaine	3.40	1.45	1.64	−0.19
Alprenolol	3.10	1.53	1.39	0.14
Etidocaine	3.22	1.55	1.49	0.06
Tetracaine	3.55	1.75	1.76	−0.01
Propranolol	3.56	1.81	1.77	0.04

[a] observed logarithm of capacity factor at 100% aqueous phase. [b] values generated from log P by eq. 3.

By omitting tocainide from eq. 2, we obtained a significant improvement in the correlation equation:

$$\log k_W^{IAM} = 0.666\ (\pm 0.091) \log P - 0.693\ (\pm 0.249) \quad (4)$$

$$n = 12 \qquad r = 0.917 \qquad s = 0.228$$

However, this correlation alone did not allow one to infer that the interactions between the phospholipids and LAs considered were uniquely lipophilicity-based. As a matter of fact, neither attractive nor repulsive additional forces could be excluded a priori, especially if they occurred in a constant extent for all the compounds. The inclusion of these compounds in eq. 3 generated a new equation for 32 compounds (plot shown in figure 3):

$$\log k_W^{IAM} = 0.775\ (\pm 0.035) \log P - 0.979\ (\pm 0.121) \quad (5)$$

$$n = 32 \qquad r = 0.971 \qquad s = 0.191$$

This relationship indicates that a mainly lipophilic mechanism governs the IAM retention of all the LAs included in eq. 5. It also indicates that phospholipids can substantially counteract the negative influence on lipophilic interactions caused by the electric charges on the basic solutes, as already observed for acidic compounds (12). However, eq. 5 was slightly worse than eq. 3 with regard to r and s statistics. This probably arises from the fact that of the LAs considered, attractive or repulsive polar extra-interactions do indeed occur and, although they play a minor role in the retention, they can disturb to a different extent the interaction, mainly lipophilicity-based, between the compounds and phospholipids.

It is worth noting that W36017 was the LA showing the highest Δ log k_w^{IAM} value (0.64), besides tocainide. Its log P value (1.23) fell markedly outside the range of log P values (2.05–5.56) considered to derive eq. 3. Therefore, we cannot exclude a priori that eq. 3 could incorrectly work, at least in part, outside this range of lipophilicity. As an example, we report that the exclusion of W36017 from the log k_w^{IAM}/log P relationship of eq. 5 yields a more significant equation (n = 31; r = 0.978; s = 0.163) and the exclusion from eq. 4 not only improves r and s statistics (n = 11; r = 0.947; s = 0.183) but also generates an equation with slope and intercept values (0.802 and −1.098, respectively) practically identical to those

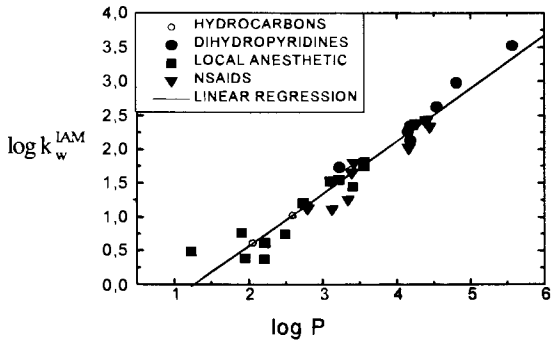

Fig. 3. Relationship between IAM chromatographic parameters (log k_w^{IAM}) and n-octanol/buffer partition coefficients (log P) for 32 structurally unrelated compounds.

of eq. 3, the latter being relative to the compounds interacting with IAM by a uniquely lipophilicity–based mechanism.

It is important to note that eq. 5 appears reasonably significant, although it includes structurally unrelated neutral, acidic, and basic molecules (neutral dihydropyridines and hydrocarbons; arylacetic and arylpropionic acids; aliphatic amines). Hence, in contrast with the HPLC parameters obtained on hydrocarbon stationary phases (19), the IAM measures of lipophilicity and log P values are on a unique scale if both nonionizable and structurally unrelated ionizable molecules are considered. Therefore, it can be inferred that when only lipophilic interactions take place between molecules of different chemical structures and phospholipids, they are effectively mimicked by the *n*-octanol/water system (log P) and *vice versa.*

Two important conclusions can be drawn from these observations:

i) When the phospholipids-analytes interactions are only lipophilicity based, they are adequately described, even for ionized molecules (basic or acidic), by intrinsic lipophilicity parameters (log P) determined by *n*-octanol/water system. In contrast, the significance of log D parameters to describe these interactions appears very poor.

ii) The usefulness of IAM values consists of their capability to detect the occurrence of polar extra-interactions, whether attractive or repulsive, with phospholipids. In fact, for ionizable compounds the occurrence of extra-interactions is not predictable a priori, as they appear related to various structural characteristics and not only to the pKa value of compounds.

Comparison Between log k_w^{IAM} Scale and Partition Coefficients on Phosphatidylcholine Membranes

In order to confirm that the log k_w^{IAM} scale for LAs actually represent a measure of partitioning in membranes, values of partitioning between aqueous buffer and multilamellar liposomes have been considered. The values, taken from the literature and measured by two different methods (20–23), are shown in Table 3.

P_{PS} values were determined by a procedure consisting of the addition of liposomes to an aqueous phase containing the analyte and in the subsequent measurement of the solute fraction remaining in the aqueous phase after centrifugation.

P_{SOL} values were determined by experiments based on the assumption that a measured effect of membrane structure is proportional to the amount of solute partitioning into the membrane. Monitoring this effect provides an estimate of the concentration of solute in both the membrane and, indirectly, in the aqueous phase. The log P calculation is thus possible.

Table 3. Values of Partition in Model Membranes[a]

Compound	log k_w^{IAM}	log P_{PS}[b]	log P_{SOL}[c]
Procaine	0.39	1.92	1.86
Prilocaine	0.62	2.04	1.98
Lidocaine	0.75	2.16	1.96
Mepivacaine	0.77	1.99	1.88
Bupivacaine	1.45	2.90	2.94
Etidocaine	1.55	3.08	3.03
Tetracaine	1.75	2.94	2.84

[a] from references 20–23.
[b] values obtained by centrifugation method.
[c] values obtained by aqueous phase saturation method.

The relationships between log k_w^{IAM} and both log P_{PS} and log P_{SOL} values were quite satisfactory:

$$\log k_w^{IAM} = 0.998\ (\pm 0.119) \log P_{PS} - 1.388\ (\pm 0.296) \quad (6)$$

$$n = 7 \qquad r = 0.966 \qquad s = 0.150$$

$$\log k_w^{IAM} = 0.917\ (\pm 0.139) \log P_{SOL} - 1.120\ (\pm 0.336) \quad (7)$$

$$n = 7 \qquad r = 0.947 \qquad s = 0.187$$

How closely the IAM chromatographic measures represent the partitioning in membranes is evident in the slopes of essentially unity in eqs. 6 and 7. This indicates an isodiscriminative behavior of the systems considered, confirming that IAM measures, performed on a monolayer of phospholipids, completely mimic the partition process on the bilayers of phosphatidylcholine model membranes. However, although the different systems for evaluation of partition in biomembranes give substantially equivalent information, the IAM method is advantageous since it is simpler to perform and more reproducible than partition methods performed on liposomes.

log k_w^{IAM} Scale for Description of Biological Activity

LAs block nerve conduction impulse by a direct interaction with voltage-gated sodium channels. The binding portion of the receptor site for these drugs is thought to consist of two amino acid residues, phenylalanine and tyrosine. They are located in transmembrane segment S_6 in domain IV of α subunit of the voltage-gated sodium channel (24).

Protonated forms of LAs are thought to access the binding site from the inner surface of the biological membrane when the Na^+ channel is in an open state. On the other hand, evidence exists that the neutral drug forms promote closed-channel block via a lipid access route to the receptor. That is, the drug pool which drives the closed channel binding step represents the neutral drug form dissolved in membrane lipid near the channel-receptor site.

An interesting correlation between lipophilicity data from theoretical calculation (CLOGP) and half-blocking doses for closed Na^+ channels was found by Courtney (15). However, this correlation applied only to amide-linked compounds, while the activities of ester- and ether-linked drug structures were strongly underestimated by their lipophilic properties.

In this study we found that phospholipid affinity (log k_w^{IAM}) scale was distinctive from the one obtained by experimental *n*-octanol/buffer partition measures (log P). Moreover, experimental lipophilicity values (log P) and the values calculated (CLOG P) did not completely match. Hence, considering the hypothesis presented above on a lipid access route to the receptor, the comparison between the different membrane affinity scales (log P and log k_w^{IAM}) for the prediction of closed Na^+ channel activity seems very interesting.

The activity data considered were the doses, corrected for ionization at pH 7.4, that are able to block half the sodium channels in well rested preparations, D'_{50} (Table 4) (15). The amount of neutral drug form at pH 7.4 (D'_{50}) within D_{50} (the total amount of drug used in experiments) can be estimated by using the pKa of each drug: $D'_{50} = D_{50}/(1 + 10^{pKa-pH})$

Table 4. Half-blocking Doses for Closed Sodium Channels

Compound	pKa	log k_w^{IAM}	pD_{50}[a]	pD_{50}^{I}[b]
GEA968	7.7	0.38	−2.94	−2.46
Procaine	9.0	0.39	−1.94	−0.33
W36017	7.4	0.49	−3.18	−2.88
Tocainide	7.8	0.53	−2.82	−2.27
Prilocaine	7.8	0.62	−3.03	−2.48
Lidocaine	7.9	0.75	−2.15	−1.53
Mepivacaine	7.6	0.77	−2.17	−1.76
Trimecaine	7.4	1.21	−1.65	−1.35
Bupivacaine	8.1	1.45	−1.52	−0.74
Alprenolol	9.6	1.53	−1.74	0.46
Etidocaine	7.7	1.55	−0.83	−0.35
Tetracaine	8.5	1.75	0.47	1.60
Propranolol	9.4	1.81	−1.59	0.41

[a] half-blocking doses calculated from data in ref. 15 and references cited therein.
[b] half-blocking doses corrected for ionization at pH 7.4.

Very poor correlation was found between pD'_{50} and log P values for the thirteen compounds considered (n = 13; r = 0.766; s = 0.914). Moreover, in contrast with the results reported by Courtney (15), who found a good correlation between pD'_{50} and CLOGP data for the amide linked compounds, the relationship we observed between the experimental log P and pD'_{50} values for this subset was also very poor (n = 9; r = 0.790; s = 0.557).

Much better correlations were observed between log k_w^{IAM} values and pD'_{50} activity data considering both the whole set of LAs and the amide subset (eqs. 8 and 9, respectively):

$$pD'_{50} = 2.033\ (\pm 0.442) \log k_w^{IAM} - 3.121\ (\pm 0.505) \quad (8)$$

n = 13; r = 0.812; 0.831

$$pD'_{50} = 1.854\ (\pm 0.240) \log k_w^{IAM} - 3.355\ (\pm 0.229) \quad (9)$$

n = 9; r = 0.946; s = 0.294

However, in eq. 8 only procaine and tetracaine appeared as outliers. In fact, by omitting only these compounds from the equation, we obtained a good correlation:

$$pD'_{50} = 2.176\ (\pm 0.234) \log k_w^{IAM} - 3.553\ (\pm 0.262) \quad (10)$$

n = 11; r = 0.952; s = 0.378

For the compounds included in eq. 10 a poorer equation was obtained if pD_{50} values were considered instead of pD'_{50} (n = 11; r = 0.889; s = 0.366). This is in agreement with the hypothesis that it is the nonionized drug forms that interact with closed channels; such discrepancy could be observed because the pD_{50} and the pD'_{50} scales were not colinear, due to the presence of the two β-blockers (alprenolol and propranolol) which have much higher pKa values than the other compounds.

These results indicate that membrane bilayer could work as reservoir of LA agents, determining their access to closed sodium channels. The partition of compounds into phospholipids appears insensitive to, or even promoted by (as in the case of tocainide), the presence of a protonated aminic function on the molecule and is adequately described by log k_w^{IAM} scale. This behavior emphasizes the role played by the amphiphilic nature of phospholipids when regulating the access of ionized compounds from outside the membrane.

On the other hand, the description of the biological effect must take into account only the neutral fraction of a half-blocking dose. This could indicate that the access route to the channel receptor site is made up of the apolar lipidic core of the membrane in which neutral forms better diffuse. However, we cannot rule out a specific lipophilic component for the binding site as an alternative or additional explanation for these results.

Procaine and tetracaine appeared relatively more potent than the other LAs in blocking closed channels, probabily due to extra-potency at the receptor site. These compounds are ester-linked and have a para-amino substituent as well. Therefore, it is not possible, from these results, to ascertain which one of these structural motifs is responsible for this effect. Moreover, we cannot exclude that the two structural features must occur simultaneously.

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