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Short communication

Chromatographic indices determined on an immobilized artificial membrane (IAM) column as descriptors of lipophilic and polar interactions of 4-phenyldihydropyridine calcium-channel blockers with biomembranes†

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Summary — A set of nine 4-phenyldihydropyridine (DHP) calcium-channel blockers including both ionizable and unionizable molecules has been examined. The chromatographic parameters log k' have been determined by HPLC on an immobilized artificial membrane (IAM) column which is a solid-phase model of fluid membranes. The influence of different percentages of organic modifier and ionic strength of the eluent on the chromatographic behaviour has been studied in order to identify the best experimental conditions modelling the in vivo interaction with phospholipids. As different ranking orders can occur under different experimental conditions, log k' values extrapolated to 100% aqueous phase (log k_w^{IAM}) have been determined. Moreover, *n*-octanol/buffer partition data at pH 7.4 and 12.5 (log $D_{7.4}$ and log P) and chromatographic data on a hydrocarbon HPLC stationary phase (log k_w^{ODS}) have been measured. Comparative studies between the experimental data, obtained for the different systems, have shown that the IAM-derived scale is distinct from the one obtained by 'conventional' lipophilic indices, because of the particular behaviour of the basic DHPs. Moreover, only IAM parameters are good descriptors of the strong interactions of the basic DHPs with biomembranes. In fact, the chromatography of neutral compounds is mainly lipophilicity dependent while a 'dual' mechanism, partition and ion-exchange, operates for basic analogues. In this case the lipophilic component is insensitive to the protonation of the basic function. Finally, receptor binding values from rat cortical brain preparations successfully correlate with log k_w^{IAM}. Hence, the biomembrane affinity of DHPs appears to be a critical factor for access to their receptor site.

HPLC / immobilized artificial membrane (IAM) / log *P* / 4-phenyldihydropyridine / biomembrane

Introduction

The 4-phenyldihydropyridines (DHPs) form an important class of drugs active as calcium-channel blockers. They are highly lipophilic molecules that bind to membrane-associated receptors. Recent reports on the mechanism of action of lacidipine, a DHP possessing a long clinical half-life, have emphasized the importance of its membrane solubility [1, 2]. In particular, a receptor model has been proposed in which the DHP binding site is a protein compartment surrounded by lipid molecules. Therefore, the lipid solubility of DHPs could represent a critical factor, governing not only the pharmacokinetic properties but also the access capability to the receptor site. Moreover, Herbette et al [3] observed that for a set of both ionized and unionized DHPs the log P scale (logarithm of the partition coefficient *n*-octanol/buffer) does not parallel the partition coefficient in a biological membrane, concluding that DHP interactions with biomembranes are complex. Different methods should be investigated in order to identify the in vitro system which best models the interactions of DHPs with biomembranes.

Conventional lipophilic indices include log P values from the shake–flask method, clog P data from theoretical calculations, and chromatographic capacity factors determined in reversed phase [4–8].

Recently, a new HPLC stationary phase material, the so-called immobilized artificial membrane (IAM), became available [9]. The IAM phase is usually comprised of monolayers of lecithin (phosphatidylcholine) wherein each lipid molecule is covalently bound to propylamine/silica; unreacted propylamine moieties can be end-capped with methylglycolate (IAM PC·MG).

The idea that IAM chromatography can be used to measure solute/membrane partition coefficients was

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†Dedicated to the memory of Profs Carlo Silipo and Antonio Vittoria

validated by Ong et al [10], who compared the retention data determined on an IAM column (k'_{IAM}) for 23 structurally unrelated compounds with their liposome partition coefficients. An excellent linear correlation was found with a slope very close to unity; this indicates that the solute partition process between the IAM-bonded phase and the aqueous mobile phase is similar to that between liposomes and the aqueous phase. Kaliszan et al [11] reported that k'_{IAM} data represent better descriptors of the hydrophobicity-affected bioactivity data than log P. Moreover good linear relationships were found between log k'_{IAM} and log P data for a set of β-adrenolitics, indicating that the retention mechanism is mostly due to lipophilic interactions. It is noteworthy that the linear correlation log k'_{IAM} versus log P values might have been observed since the basic homologues examined showed a similar ionization degree in the experimental conditions. In fact, Ong et al [10] pointed out that good correlations between log P and IAM chromatographic parameters are usually found for homologous series of hydrophobic solutes, which interact mainly with the nonpolar part of the lipid phase. In contrast, for chemical structures that have polar functional groups that can interact with a lipid head group, the correlation of log P with IAM chromatographic parameters is poor. These observations indicate that for molecules supporting polar functional groups, the interactions with phospholipids can be complex and not completely understood.

In this study the conventional lipophilic indices, log P and clog P, of nine DHPs (table I) have been determined and compared with the HPLC indices on octadecylsilyl (ODS) (log k'_{ODS}) and IAM phases. Although the set of DHPs we have considered are constituted of structurally related compounds, it includes both unionizable and ionizable analogues (amlodipine and nicardipine are basic molecules supporting a primary and tertiary aminic function respectively). Such a set of compounds can stress the differences of the interaction mechanisms operating in the different systems considered. In particular, IAM chromatography could provide an interaction scale distinctive from the one obtained by log P or log k'_{ODS} indices [12, 13]. On the other hand, the significance of IAM chromatographic behaviour of neutral and basic analogues is strictly related to the experimental conditions. We thus underline that the chromatographic behaviour of neutral and basic analogues can be affected in different ways by the eluent ionic strength and fraction of organic modifier. As a consequence, different ranking orders can occur under different experimental conditions. Hence, the study of the influence of different experimental conditions on the IAM chromatographic behaviour of DHPs could contribute to an insight into not only the chromatographic retention mechanisms, but also into the in vivo interaction with biomembranes. Finally, we have verified the capability of the IAM interaction scale for describing some biological phenomena.

Experimental protocols

Chromatographic system

A liquid chromatograph Model 600E (Waters-Millipore, Milford, MA, USA) equipped with a Model 7125 Rheodyne injection valve (fitted with a 20 μL loop) and a Model 486 UV detector (Waters) set at 238 nm was used. The stainless steel columns were a Spherisorb 5 ODS-2 (4.6 x 250 mm) (Phase Separation, Clwyd, UK) and an IAM PC.MG column (4.6 x 150 mm) (Regis Chemical Company, Morton Grove, IL, USA). The chromatograms were recorded by a Data Module Model 746 (Millipore).

Materials

All samples were obtained from a commercial source. All chemicals were of analytical grade and used without further purification.

Chromatographic conditions

For IAM chromatography the eluents were mixtures of acetonitrile and phosphate buffer saline (PBS) 0.01–0.10 M at pH 7.0 in different percentages at a flow rate of 1.0 or 2.0 mL/min. For ODS chromatography the mobile phases were aqueous methanol, in various proportions buffered with 3-morpholinopropanesulphonate (0.02 M, adjusted to pH 7.0 with hydrochloric acid) and containing *n*-decylamine (0.2%, v/v) in order to mask the silanol sites on the stationary phase [14–16]. The eluent flow rate was 0.8 mL/min. The aqueous portion of eluents were filtered by HA filters (Millipore). The eluent mixtures were obtained directly from the chromatographic apparatus by mixing at low pressure the organic modifier and the aqueous phase previously degassed by bubbling helium. The chromatography was carried out at room temperature. DHPs were dissolved in methanol (ca 10^{-3} M); 20 μL samples were injected into the chromatograph.

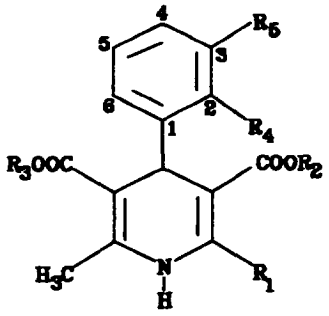
Chromatographic retention data are expressed by the logarithm of the capacity factor, log k', defined as log k' = log $[(t_r - t_0)/ t_0]$ where t_r and t_0 are the retention times of the drug and a non-retained compound (methanol), respectively.

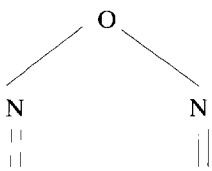
Log P evaluation

Partition coefficients (P) have been determined according to the shake–flask procedure. *n*-Octanol was used as the lipophilic phase whereas the aqueous portions were a phosphate buffer at pH 7.4 (Na_2HPO_4 9.70 g and KH_2PO_4 1.65 g in 1 L water) and a buffer at pH 12.5 (KCl 2.85 g and NaOH 0.53 g in 1 L water). Some of the examined DHPs (nifedipine and nisoldipine) are very sensitive to light, forming their photodegradation derivatives quickly in solution [18–22]. Since this phenomenon can affect the shake–flask measurements, we took care to avoid exposure to light (all reaction vessels were wrapped with aluminium foil and all operations were performed under sodium light). The quantitation after partition was performed by HPLC method suitable for detecting the possible presence of photodegradation products [17].

All reported values of log k' and log P are the averages of at least three measurements.

Table I. Lipophilic parameters for 4-phenyldihydropyridines.



Compound	R_1	R_2	R_3	R_4	R_5	*clog P* [b]	*log P* [c] *(log $D_{7.4}$)*	*log* k_w^{ODS} [e]
Nifedipine	CH_3	CH_3	CH_3	NO_2	H	2.35	3.22	2.45
Isradipine[a]	CH_3	CH_3	$CH(CH_3)_2$			3.14	4.18	3.10
Nitrendipine	CH_3	CH_3	C_2H_5	H	NO_2	2.96	4.15	3.17
Nimodipine	CH_3	$CH(CH_3)_2$	$CH_2CH_2OCH_3$	H	NO_2	3.09	4.18	3.25
Nisoldipine	CH_3	CH_3	$CH_2CH(CH_3)_2$	NO_2	H	3.81	4.53	3.26
Felodipine	CH_3	CH_3	C_2H_5	Cl	Cl	4.52	4.80	3.82
Lacidipine	CH_3	C_2H_5	C_2H_5	$COOC(CH_3)_3$ | $CH=CH$	H	5.31	5.56	4.59
Nicardipine	CH_3	CH_3	$(CH_2)_2NCH_2C_6H_5$ (with CH_3 branch)	H	NO_2	4.22	4.96[d] (3.72)	3.36
Amlodipine	CH_2 | $O(CH_2)_2NH_2$	C_2H_5	CH_3	Cl	H	2.78	3.30[d] (1.83)	1.33

[a]In this case there is a single bond between C_2 and C_3 in the general formula; [b]values calculated with C-QSAR program, version 1.83, Biobyte Corp, Claremont, CA, USA; [c]logarithm of *n*-octanol/buffer pH 7.4 partition coefficient determined by the shake-flask method. The log *P* values are the average of three determinations; the 95% confidence interval associated to each log *P* value was never greater than 0.04; [d]logarithm of *n*-octanol/buffer pH 12.5 partition coefficient determined by the shake-flask method; [e]logarithm of the capacity factor extrapolated to 100% aqueous phase obtained on ODS column.

Results and discussion

Table I summarizes the log P values measured by the shake–flask method using n-octanol/phosphate buffer at pH 7.4 as the partitioning system. Amlodipine and nicardipine are weak bases (pK_a = 9.1 and 6.5 respectively) [17] and their partition between n-octanol and buffer at pH 7.4 (indicated as log $D_{7.4}$) is relative to a mixture of ionized and unionized species. In this case the log P value, relative to the unionized form, has been determined at a pH value of 12.5.

The measured log P values were found to be higher than the calculated values, clog P, and are interrelated by the following equation:

$$\log P = 0.747\ (\pm 0.095)\ \text{clog}\ P + 1.650\ (\pm 0.351) \quad (1)$$
$$n = 9;\ r = 0.948;\ s = 0.257.$$

In this and 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 estimate.

The log P value measured for lacidipine is in agreement with the previously determined experimental value [23] and therefore the differences found between calculated and measured partition coefficients of DHPs indicate that the clog P method calculation improperly handles this type of structure.

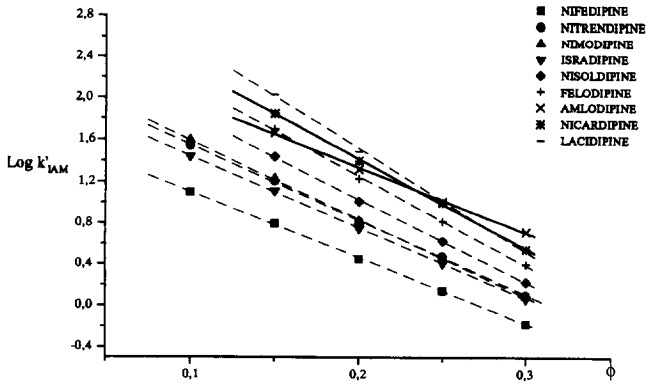


Fig 1. Plots of logarithm of capacity factors determined on an IAM column (log k'_{IAM}) at different fractions of acetonitrile (φ). - - - - Neutral DHPs; —— basic DHPs. Eluent: acetonitrile/PBS 0.1 M pH 7.0; flow rate 2.0 mL/min.

Relationship between log k'_{IAM} and φ

The values of log k' on the IAM column (log k'_{IAM}) were determined at various acetonitrile fractions (φ). A linear relationship between log k'_{IAM} and φ was found for all DHPs over the examined range of eluent composition (fig 1). The logarithm of the capacity factors extrapolated to 100% aqueous phase (log k_w^{IAM}) and the parameters of the regression equations are reported in table II (equations (2–10)).

Table II. Relationship between the capacity factors determined on IAM column (log k'_{IAM}) and fraction of acetonitrile (φ).
$\log k'_{IAM} = a\varphi + \log k_w^{IAM}$

DHP	*log k_w^{IAM}*	*a*	*r*	*s*	*n*	*Equation*
Nifedipine	1.74 (±0.02)	–6.38 (±0.08)	0.9998	0.0130	5	(2)
Amlodipine	2.59 (±0.04)	–6.26 (±0.16)	0.9994	0.0178	4	(3)
Nitrendipine	2.27 (±0.02)	–7.22 (±0.10)	0.9997	0.0162	5	(4)
Isradipine	2.13 (±0.01)	–6.88 (±0.05)	0.9999	0.0073	5	(5)
Nimodipine	2.35 (±0.02)	–7.54 (±0.09)	0.9998	0.0140	5	(6)
Nisoldipine	2.63 (±0.03)	–8.02 (±0.14)	0.9997	0.0153	4	(7)
Felodipine	2.98 (±0.04)	–8.62 (±0.19)	0.9995	0.0209	4	(8)
Nicardipine	3.14 (±0.03)	–8.60 (±0.14)	0.9997	0.0158	4	(9)
Lacidipine	3.52 (±0.04)	–10.00 (±0.19)	0.9996	0.0212	4	(10)

n denotes the number of molecules considered to derive 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. Eluent: acetonitrile/phosphate buffer 0.1 M pH 7.0; flow rate 2 mL/min.

The regression lines of relationships log k'_{IAM} versus φ values for the basic molecules intersect the lines relative to the neutral compounds (fig 1) indicating that a different interaction mechanism is involved in their retention on the IAM phase. It is evident from the data in figure 1 that differences in elution order occur under different percentages of organic modifier. Hence, normalization of the experimental values to 100% aqueous phase must be performed to avoid the obtention of fictitious interaction scales.

Previous chromatographic studies on hydrocarbonaceous phases [24] showed that methanol should be preferred to acetonitrile as organic modifier for the extrapolation to 100% aqueous phase of log k' values. In fact, the use of methanol was reported to produce a linear regression of log k' versus the fraction of organic modifier, while parabolic relationships were found for acetonitrile-containing systems. However, in spite of the difference in the isocratic log k' values and in their relationship with the fraction of organic modifier, the extrapolation to 100% water yielded practically identical log k_w values for both eluent systems. We could not obtain chromatographic data on the IAM column from methanol-containing eluents, as this organic modifier is reported to degrade phospholipids. However, we observed very good linear regressions in the acetonitrile–water systems (see correlation coefficients in table II). Because our values of log k'_{IAM} lie in a water-rich concentration region, probably only the linear part of a parabolic relationship is detectable [24].

Relationship between log k_w^{IAM} and log P

A good linear relationship was found between log k_w^{IAM} and log P values for all DHPs but amlodipine:

$$\log k_w^{IAM} = 0.826\ (\pm 0.076) \log P - 1.078\ (\pm 0.341) \quad (11)$$
$$n = 8;\ r = 0.976;\ s = 0.139.$$

A poor relation equation is obtained if considering for nicardipine the log $D_{7.4}$ instead of log P ($r = 0.731$, $s = 0.433$). Moreover, by including amlodipine in eq (11), very poor linear relationships are found regressing log k_w^{IAM} versus both its log P or log $D_{7.4}$ ($n = 9$, $r = 0.842$, $s = 0.317$ and $n = 9$, $r = 0.459$, $s = 0.522$, respectively). This means that the interaction of neutral DHPs with the IAM phase is mainly lipophilicity based, while a different mechanism is involved in the interaction of the basic compounds. In fact, it is surprising that the log k_w^{IAM} of nicardipine is predicted much better from the log P ('intrinsic' partition coefficient measured at pH 12.5) than from log $D_{7.4}$, although the latter takes into account protonation phenomena occurring at a pH value very close to that of the HPLC experimental conditions. Therefore the interaction between nicardipine and the IAM phase appears insensitive to the partial protonation of its basic function perhaps as a consequence of a 'shielding effect' of polar head groups of phospholipids which could accommodate the polar moiety of the solute.

The measured log k_w^{IAM} for amlodipine is much higher than the values calculated from both log $D_{7.4}$ and log P by equation (11) (table III). This indicates a much higher affinity for phospholipids of amlodipine compared with an isolipophilic neutral molecule. The observed behaviour could arise from an extra interaction (probably a polar interaction) occurring between amlodipine and polar head groups of phospholipids. This could be feasible because amlodipine is fully protonated at pH 7.0 and its basic function does not support bulky substituents. This view is in agreement with the partition values of amlodipine between phospholipid vescicles and aqueous buffers [25]. In fact, the vescicle partition values were found to be higher than log P in a pH range from 7.0 (complete protonation of amlodipine) to 11.0 (negligible degree of protonation). Moreover, the partitioning process in vescicles was practically insensitive to the pH variations of the buffer.

Because the log k_w^{IAM} values for the basic substances result from a 'dual' retention mechanism, further information about the different components of interaction could be gained by analysing the slope values of log k'_{IAM} versus φ relationships. The slope values actually result mainly from the interaction solute/stationary phase, more sensitive to the percentage of organic modifier [24].

Relationship between log k_w^{IAM} and slope

For neutral compounds a positive correlation is found between the slope and the intercept (log k_w^{IAM}) of the log k'_{IAM} versus φ relationships (values reported in table II):

$$\log k_w^{IAM} = -0.482\ (\pm 0.022)\ \text{slope} - 1.249\ (\pm 0.178) \quad (12)$$
$$n = 7;\ r = 0.995;\ s = 0.067.$$

Table III. Observed and calculated log k_w^{IAM} values for basic DHPs.

DHP	*log P (log $D_{7.4}$)*	*log $k_w^{IAM}{}_{cal}$* [a]	*log $k_w^{IAM}{}_{obs}$* [b]
Amlodipine	3.30 (1.83)	1.66 (0.48)	2.59
Nicardipine	4.96 (3.72)	2.99 (1.99)	3.14

[a]Values generated from log P (or log $D_{7.4}$) by eq (11); [b]observed logarithm of capacity factor extrapolated to a 100% aqueous phase.

In a study by Reymond et al [24], performed on an ODS column with methanol as organic modifier, the linear regression of log k_w versus slope yielded a new slope of value ca –1.0. This value was thought to be indicative of a common property exhibited by solutes. Equation (12) shows a slope of value ca –0.5, although all neutral DHPs show a common retention mechanism, lipophilicity dependent (equation (11)). We believe that the value of this slope is a consequence of the capability of φ to affect the extent of the hydrophobic expulsion of the solute from the mobile phase. Therefore, because acetonitrile is a stronger organic modifier than methanol, the same change in the percentage of organic modifier will affect much more extensively the hydrophobic expulsion process in the IAM/acetonitrile system than in the ODS/methanol system. Equation (12) implies that for neutral compounds the regression lines converge to a common point.

Including nicardipine in the relationship between log k_w^{IAM} and slope, a new regression equation is obtained with only slightly worse values of r and s (r = 0.985 and s = 0.108). This occurs because the difference between the experimental log k_w^{IAM} value and the value predicted from the slope by equation (12) is very slight and of the same order of magnitude as the standard error of the estimate. On the other hand, in deriving equation (12), the amlodipine fit was extremely poor, indicating that its interaction with the IAM phase is even further from a merely partition-based mechanism than for nicardipine.

However, the slope values for all compounds, including amlodipine, are a function of the respective log P values as shown by the following equation:

$$\text{slope} = -1.559\ (\pm 0.154) \log P - 0.991\ (\pm 0.676) \quad (13)$$
$$n = 9;\ r = 0.967;\ s = 0.329.$$

This equation implies that increasing the amount of organic modifier affects the log k'_{IAM} in a measure directly related to the lipophilicity of the compound as it is estimated in the octanol–water system. Hence, the slope values can be considered as a measure of the lipophilic interaction DHP–IAM. If so, the lipophilic interaction of amlodipine and nicardipine appears to be insensitive to the protonation of the basic function. In fact, their log P values predict the slope much better than their log $D_{7.4}$ (r = 0.798, s = 0.782). In contrast, the polar component seems to be constant in the considered range of φ. The regression lines of basic DHPs therefore appear shifted upward in figure 1 because the values of log k'_{IAM} at every organic modifier fraction consist of both a variable lipophilicity-based component and a constant polar component.

The occurrence of ion-pairs in the retention mechanism of amlodipine and nicardipine on IAM can be excluded. An increase of buffer molarity produces a decrease of their log k'_{IAM} (fig 2). Obviously, the chromatographic behaviour of neutral DHPs was practically insensitive to the variation of ionic strength of eluent.

Comparison between chromatographic behaviour on ODS and IAM columns

The log k' values on the ODS column (log k'_{ODS}) have been determined at various methanol fractions ($0.50 \leq \varphi' \leq 0.95$) and the values extrapolated to 100% aqueous phase have been calculated (log k_w^{ODS}) (table I). For all compounds a linear relationship between log k'_{ODS} and φ' was observed over the range of eluent composition examined.

Both the log k_w^{ODS} and the slope values of relationships log k'_{ODS} versus φ' correlate well with log $D_{7.4}$ values

$$\log k_w^{ODS} = 0.823\ (\pm 0.078) \log D_{7.4} - 0.162\ (\pm 0.322) \quad (14)$$
$$n = 9;\ r = 0.970;\ s = 0.231.$$

$$\text{slope} = -0.488\ (\pm 0.367) \log D_{7.4} - 0.840\ (\pm 0.089) \quad (15)$$
$$n = 9;\ r = 0.963;\ s = 0.264.$$

It is important to remember that the performed chromatographic conditions allow only a partition-based retention mechanism [24]. Therefore equations (14) and (15) demonstrate that the repulsion of charged species from a hydrocarbonaceous stationary phase is adequately accounted for by the parameter log $D_{7.4}$. By contrast, on the IAM phase the log $D_{7.4}$ of basic compounds fail to predict the slope values, which are instead predictable from the intrinsic lipophilicity parameter, log P. Therefore, the overall interaction of amlodipine and nicardipine on IAM does not

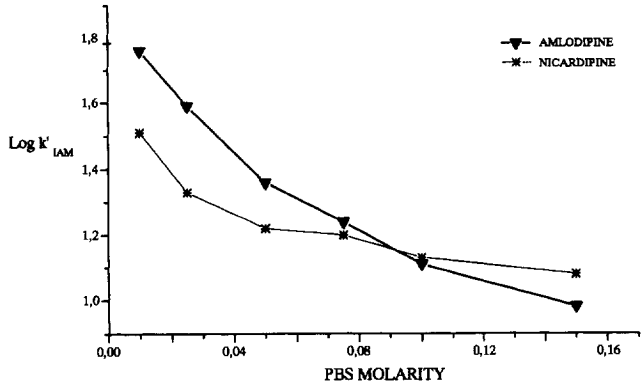


Fig 2. Plots of logarithm of capacity factors determined on IAM column (log k'_{IAM}) versus PBS molarity for amlodipine and nicardipine. Eluent: acetonitrile/PBS pH 7.0 (25:75 v/v); flow rate 1.0 mL/min.

appear as the result of two opposing effects, ie, electrostatic attraction and normal repulsion of charges from regions of lower polarity than water. Instead, the polar moieties of phospholipids appear to give rise simultaneously to two different effects: i) a polar extrainteraction with charged moieties of solutes (resulting in higher log k_w^{IAM} than expected); and ii) a neutralization of interference of the charges on lipophilic interaction (resulting in slope values that are collinear to the log P).

Thus, on IAM, a unique retention mechanism for amlodipine and nicardipine can be hypothesized, consisting of a simultaneous accommodation of their polar and lipophilic moieties respectively in the polar head groups and apolar chain (miristoyl residue) of phospholipids. This cooperative retention mechanism is also indicated by the good symmetry of their chromatographic peaks (asymmetry factor very close to unity) whereas, chromatographic runs on the ODS phase performed with eluents without n-decylamine (interactions with free silanol residues on silica core are possible) showed an evident peak-tailing phenomenon.

Log k_w^{IAM} as descriptors of biological data

Herbette et al [3] observed a discrepancy between log P and the biological membrane–water partition coefficient (P_m) for amlodipine. In fact, the log P of amlodipine was nearly an order of magnitude lower than that of nimodipine while its P_m was over threefold higher (their log P_m values were 4.30 and 3.80 respectively), ie, the log P_m of amlodipine was 13% higher than that of nimodipine. These observations suggested that the interactions of DHPs with biomembranes can be complex and not modelled by n-octanol/buffer systems.

Therefore a comparison between the log k_w^{IAM} scale and biomembrane affinity seems very interesting since the IAM scale for the considered DHPs is distinctive from that obtained with log P values, because of the different behaviour of the basic substances in the two systems. The data we obtained by IAM chromatography are actually in agreement with the results reported above; the k_w^{IAM} observed for amlodipine is 10% higher than that of nimodipine (2.59 and 2.35 respectively). Thus, the affinity scale obtained from IAM chromatography seems to be a better descriptor of the interaction DHP/biomembranes than the conventional lipophilic indices when comparing the behaviour of basic and neutral analogues.

The usefulness of membrane affinity indices (log P, log k_w^{ODS} and log k_w^{IAM}) is usually ascribed to their capability to predict the pharmacokinetics and mainly the absorption of drugs, but in some cases the capability of access to the receptor site can also strongly depend on these parameters.

The examined class of DHPs is a case in point. We have found a good correlation between log k_w^{IAM} (or log P) and receptor binding values (k_i) relative to four neutral DHPs (nifedipine, nitrendipine, nimodipine and nisoldipine) (table IV) measured on protein preparations from rat cortical brain [26]: $k_i = IC_{50}/(1 + LC/k_d)$ in which LC is ligand concentration, k_d its dissociation constant, and IC_{50} the concentration of drug causing 50% inhibition of [^{3}H]-nimodipine specific binding. The k_i values can thus be considered as indices of an overall DHP effect, including both their specific receptor binding and biomembrane permeation.

The log $1/k_i$ values correlate well with both log k_w^{IAM} and log P parameters, as shown in equations (16) and (17):

$$\log 1/k_i = 1.581\ (\pm 0.225) \log k_w^{IAM} - 3.621\ (\pm 0.511) \quad (16)$$
$n = 4$; $r = 0.980$; $s = 0.145$,

$$\log 1/k_i = 1.039\ (\pm 0.181) \log P - 4.244\ (\pm 0.732) \quad (17)$$
$n = 4$; $r = 0.971$; $s = 0.176$.

Although there were not enough k_i data reported to derive meaningful relationships with log k_w^{IAM} and log P, these results suggest that membrane solubility may constitute the crucial factor that allows the specific binding DHP/receptor.

From the results obtained so far it is possible to infer that for the neutral DHPs all the considered systems give satisfactory and parallel descriptions of biomembrane interaction. However, only the IAM parameter gives an adequate evaluation of biomembrane partition for a basic compound such as amlodipine.

Conclusions

The retention scale obtained with IAM system for considered DHPs is distinctive from the one obtained with log P values. The present study confirms that

Table IV. Receptor binding data (k_i) for neutral DHPs.

DHP	k_i[a]	*log k_w^{IAM}*	*log P*
Nifedipine	7.0	1.74	3.22
Nimodipine	1.2	2.35	4.18
Nitrendipine	0.93	0.27	4.15
Nisoldipine	0.24	2.63	4.53

[a] $k_i = IC_{50}/(1 + LC/k_d)$ in which LC is ligand concentration, k_d its dissociation constant, and IC_{50} the concentration of causing 50% inhibition of [^{3}H]-nimodipine specific binding [26].

IAM phase interacts with unionizable apolar compounds by a partition-based mechanism. Therefore IAM or ODS chromatographic indices and log *P* *n*-octanol/buffer are collinear parameters for quantitative structure–activity relationship studies. On the other hand, a different behaviour was observed when all the DHPs were considered, including the basic molecules. The affinity of the basic DHPs with the IAM phase is much stronger than that expected by the lipophilic indices and is in agreement with the data of partition with both biomembranes and liposomes. This means that the behaviour of bases on IAM is produced from specific interactions with phospholipids and is not a consequence of secondary interactions with silica core. Therefore the IAM parameter provides an overall affinity scale which can better reflect the different biological effects for a mixed set of compounds including ionizable and unionizable analogues, such as the examined DHPs.

A main role in the original retention mechanism of the basic DHPs on IAM is played from the polar head group of lecithines. This seems able not only to establish a polar additive extra-interaction with protonated basic functions of solutes but also to counteract their influence on the lipophilic interaction. The interaction between the basic compounds examined and the IAM phase can be supposed to be the result of two simultaneous phenomena: i) the interaction between the apolar chain of phospholipids (miristoyl residue) and the lipophilic moiety of amlodipine and nicardipine; and ii) the accommodation of the polar basic group of the solute in the water-rich concentration region surrounding the polar head groups of phospholipids. However, further studies using other basic compounds are needed to support this hypothesis and to gain an insight into the possible role of molecular geometry and pK_a of solutes in the interaction with phospholipids.

Finally, the access capability of DHPs to their receptor site has found to be strongly dependent on phospholipid affinity parameters. This should be taken into account when considering new drug candidates of dihydropyridine class.

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References

1 Micheli D, Ratti E, Toson G, Gaviraghi G (1991) *J Cardiovasc Pharmacol* 17 (suppl 4), s1–s8
2 Zanchetti A (1994) *Lacidipine: A Clinical Monograph.* ADIS International, Chester, 6–7
3 Herbette LG, Gaviraghi G, Tulenko T, Preston Mason R (1993) *J Hypert* 11 (suppl 1), s13–s19
4 Leo AJ, Hansch C, Elkins D (1971) *Chem Rev* 71, 525–616
5 Pomona College Medicinal Chemistry Project (1983) *Log P and Parameter Database: A Tool for the Quantitative Prediction of Bio-activity.* Comtex Scientific Corporation, New York
6 Leo AJ (1990) In: *Comprehensive Medicinal Chemistry* (Hansch C, Sammes PG, Taylor JB, eds), Pergamon Press, New York, vol 4 (Ramsden CA, volume ed) 295–319
7 Leo AJ (1993) *Chem Rev* 93, 1281–1306
8 Snyder LR, Kirkland JJ (1979) *Introduction to Modern Liquid Chromatography*, John Wiley, New York
9 Turnhofer H, Schnabel J, Betz M, Lipka G, Pidgeon C, Hauser H (1991) *Biochim Biophys Acta* 1064, 275–286
10 Ong S, Liu H, Qiu X, Bhat G, Pidgeon C (1995) *Anal Chem* 67, 755–762
11 Kaliszan R, Nasal A, Bucinski A (1994) *Eur J Med Chem* 29, 163–170
12 Nasal A, Sznitowska M, Bucinski A, Kaliszan R (1995) *J Chromatogr* A 692, 83–89
13 Pidgeon C, Ong S, Liu H et al (1995) *J Med Chem* 38, 590–594
14 Snyder LR, Glajch JL, Kirkland JJ (1988) *Practical HPLC Method Development*, Wiley-Intersciences, USA, 56–61
15 Paesen J, Claeys P, Roets E, Hoogmartens J (1992) *J Chromatogr* 630, 117–122
16 Stoev G, Uzonov D (1992) *J Liq Chromatogr* 15, 3097–3114
17 Barbato F, Cappello B, Grumetto L, Morrica P (1993) *Il Farmaco* 48, 417–426
18 Testa R, Dolfini E, Reschiotto C, Secchi C, Biondi PA (1979) *Il Farmaco, Ed Pr*, 34, 463–473
19 Tucker FA, Minty PSB, MacGregor GA (1985) *J Chromatogr* 342, 193–198
20 Logan BK, Patrick KS (1990) *J Chromatogr* 529, 175–181
21 Jakobsen P, Mikkelsen EO, Laursen J, Jensen F (1986) *J Chromatogr* 374, 383–387
22 Barbato F, Grumetto L, Morrica P (1994) *Il Farmaco* 49, 461–466
23 Craig PN (1990) In: *Comprehensive Medicinal Chemistry* (Hansch C, Sammes PG, Taylor JB, eds), Pergamon Press, New York, vol 6 (Drayton CJ volume ed)
24 Reymond D, Chung GN, Mayer JM, Testa B (1987) *J Chromatogr* 391, 97–109
25 Austin RP, Davis AM, Manners CN(1995) *J Pharm Sci* 84, 1180–1183
26 Bellemann P (1986) In: *Pharmacochemistry Library* (Nauta WT, Rekker RF, eds) Elsevier Science Publishers BV, Amsterdam, vol 9 (Harms AF, volume ed), 23–46