Lipophilicity Behaviour of the Zwitterionic Antihistamine Cetirizine in Phosphatidylcholine Liposomes/Water Systems

Georgette Plemper van Balen,[1] Giulia Caron,[1,2] Giuseppe Ermondi,[3] Alessandra Pagliara,[1] Teresa Grandi,[1] Géraldine Bouchard,[1] Roberta Fruttero,[2] Pierre-Alain Carrupt,[1] and Bernard Testa[1,4]

Received January 22, 2001; accepted February 12, 2001

Purpose. The partitioning of cetirizine in a phosphatidylcholine liposomes/water system was compared with that of hydroxyzine and acrivastine to gain insight into the mechanisms of interaction of its various electrical species with membranes.

Methods. The lipophilicity profiles of the compounds were obtained from equilibrium dialysis and potentiometry, and compared with changes in NMR relaxation rates.

Results. The neutral form of hydroxyzine interacted mainly via hydrophobic interactions with the bilayer lipid core of the membrane, whereas for the cationic form both hydrophobic and electrostatic interactions were involved. Zwitterionic and anionic cetirizine were less lipophilic than its cation, which behaved like the corresponding species of hydroxyzine. Zwitterionic cetirizine interacted more by weak electrostatic interactions with the polar headgroups of phospholipids than by hydrophobic interactions with the membrane interior. The lipophilicity of its anion reflected the balance of repulsive electrostatic interactions between the carboxylate and phosphate groups and the hydrophobic interactions with the lipid core.

Conclusion. The study confirms that various mechanisms influence the interaction of solutes with liposomes. Combining experimental techniques and using suitable reference compounds proves useful.

KEY WORDS: cetirizine; phosphatidylcholine liposomes; equilibrium dialysis, potentiometry; NMR; distribution profiles; lipophilicity.

INTRODUCTION

Cetirizine (Fig. 1) is a well known second-generation long-acting H_1-antagonist (1). Recently, Pagliara *et al.* (2) examined its acid-base behaviour (Fig. 2) and determined its pH-partitioning profile in isotropic solvent systems such as *n*-octanol/water (Fig. 3A) and dodecane/water. The physicochemical characteristics of the zwitterion, which predomines at physiological pH, render cetirizine very different from classical antihistamines. In particular, charge neutralization by intramolecular interactions may come into play. To validate this hypothesis, molecular dynamics and NMR spectroscopy were used to explore the property space and conformational behaviour of cetirizine and to attribute the two basic pK_a to their respective nitrogen (3). Partial intramolecular charge neutralization was thus demonstrated. It has also been suggested that folded conformers of zwitterionic cetirizine dominate its partitioning behaviour.

Beside isotropic systems, it becomes of increasing relevance to investigate the partitioning behaviour of drugs in anisotropic systems such as phosphatidylcholine liposomes/water. In fact, it has been demonstrated that the partition coefficient of ionized ligands in lipidic membranes differs significantly from that in octanol, as ionized species may strongly associate with the zwitterionic phospholipid by means of electrostatic interactions (4–7).

In this study, results from equilibrium dialysis and ^{1}H-NMR spectroscopy experiments were combined. Dialysis experiments were carried out to get a general idea of the distribution profile of cetirizine in a liposomes/water system. The NMR experiments, on the other hand, served to investigate in more detail the interactions between the various electrical species of the drug and the liposomal bilayer.

For comparison purposes, hydroxyzine and acrivastine (Fig. 1) were also included. Hydroxyzine (Fig. 1), the parent drug of cetirizine lacking an acidic group, was included (3) because of its structural resemblance with cetirizine. Acrivastine (Fig. 1) is a highly rigid zwitterionic compound presenting a lipophilicity profile in *n*-octanol/water that is different from that of cetirizine (8).

MATERIALS AND METHODS

Compounds and Reagents

Cetirizine·2HCl and hydroxyzine·2HCl were kindly donated by Dr. Jean-Pierre Rihoux (UCB Pharma, Braine-l'Alleud, Belgium). Acrivastine was kindly provided by The Wellcome Foundation Ltd (Dartford, UK).

Egg phosphatidylcholine (PhC), grade I, was purchased from Lipid Products (South Nutfield, Surrey, UK). The Extruder type T001 and T002 were purchased from Sirius Analytical Instruments Ltd (East Sussex, UK). The polycarbonate filters with pores of 0.1 μm were obtained from Osmonics (Livermore, CA). The dialysis membranes with a cutoff point of 10,000, the drive unit Type GD 4/90, and the Macro 1, 1.0 mL dialysis cells were obtained from Dianorm GmbH (Munich, Germany). The phospholipids enzymatic colorimetric test MPR2 691844 was purchased from Boehringer Mannheim GmbH (Mannheim, Germany).

Anhydrous disodium hydrogen phosphate, potassium dihydrogen phosphate and Triton X-100 were purchased from Fluka AG (Buchs, Switzerland) and potassium chloride from Merck AG (Dietikon, Switzerland). Sephadex G-50 was obtained from Pharmacia (Uppsala, Sweden) and calcein (3,5-dihydroxy-2,3-bis[N,N′-di(carboxymethyl)methyl]fluorescein) was purchased by ICN Biomedicals Inc. (Costa Mesa, CA).

Preparation and Characterization of the Liposomal Suspensions

Phosphatidylcholine liposomes were prepared by the extrusion method (9). In general, PhC was dried from chloro-

[1] Institut de Chimie Thérapeutique, Section de Pharmacie, Université de Lausanne, CH-1015 Lausanne, Switzerland
[2] Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Via Giuria 9, I-10125 Torino, Italy
[3] DISCAFF, Università del Piemonte Orientale, Viale Ferrucci 33, I-28100 Novara
[4] To whom correspondence should be addressed. (e-mail: bernard.testa@ict.unil.ch)

0724-8741/01/0500-0694$19.50/0

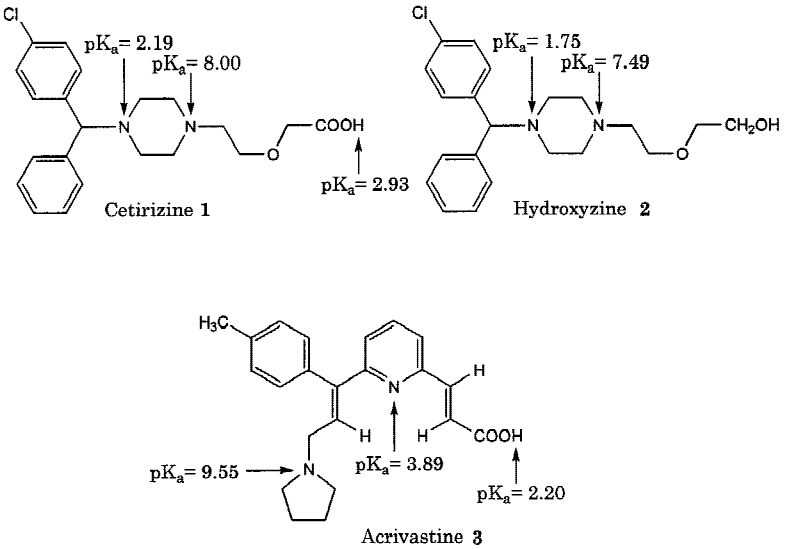

Fig. 1. Chemical structures of compounds under study.

form/methanol solutions to a thin layer in a round flask. After overnight storage at room temperature under nitrogen and in a dessicator, the lipids were hydrated with an aqueous phase to form multilamellar vesicles (MLV). This phase was either (a) a phosphate buffer of pH 7.4 (PBS) (2 mM KH_2PO_4, 8 mM Na_2HPO_4) and 0.15 M KCl (the normal equilibrium dialysis experiments), or (b) the same as (a) but with 0.37 mmol calcein for each 0.5 g of PhC (the membrane leakage experiments), or (c) 0.15 M KCl (the potentiometry experiments), or (d) D_2O (the ^{1}H-NMR spectroscopy experiments). In order to prevent oxidation of the lipids, all aqueous solutions were purged with nitrogen for at least 30 minutes.

The MLV preparations were treated by 5 cycles of freeze and thaw in liquid nitrogen and at 37°C (10). Large unilamellar vesicles (LUV) were prepared by extrusion of the MLV preparations 15 times through 0.1 μm polycarbonate filters by means of an extruder (9,11). The resulting suspensions were stored under nitrogen at 4°C for at least 24 hours before use in order to enable the spontaneous repair of possible small defects in the bilayer (9,11).

In case (b) the un-encapsulated calcein was removed by gel filtration of the liposomal suspension over a Sephadex G-50 column equilibrated with the phosphate buffer, as described under (a) (12–15). The fractions obtained were only pooled if their fluorescence after breakdown of the liposomes was similar. The breakdown of the liposomes was provoked by sonication of 3 mL of a 1000-fold diluted suspension with 30 μL 10% Triton X-100 at room temperature during 10 minutes (12–15). To measure the fluorescence, a LS-3B Perkin-Elmer fluorescence spectrometer was used (excitation at 490 nm, emission at 510 nm) (12,13,15).

The resulting liposome size distributions were measured with a Malvern Zetasizer 3 (Malvern Instruments, Worcestershire, UK). The phosphatidylcholine liposomes had a diameter of about 120 nm, which was stable during the partition experiments within the pH range 2 to 11 (10).

The concentration of phospholipids in the resulting opalescent stock suspension was determined by the use of a phospholipid enzymatic colorimetric test (16,17). The lipid concentration was determined by enzymatic breakdown of 20 μL of the liposomal suspension in 3 mL of a buffer solution containing the enzyme, followed by the quantification of the chromophore so obtained by measurement of its absorbance at 500 nm (16,17). Usually, the concentrations ranged from 80 to 100 mg/mL. For the calcein-containing liposomes, the test was slightly changed. In this assay, the absorbance of calcein present in the liposomes had to be taken into consideration. Therefore, 30 μL 10% Triton X-100 were added to a mixture of 2970 μL of PBS and 20 μL of liposomal suspension to cause breakdown of the liposomes and to release calcein. After sonication for 10 minutes, the absorbance of the suspension was measured. A solution of 30 μL 10% Triton X-100 and 2970 μL PBS served as a blank. Thus, the lipid concentrations in the colorimetric assay and the control were the same, which permitted by simple deduction to correct for calcein absorbance and to determine the lipid concentration.

The liposomal suspensions were stored under nitrogen at 4°C.

Equilibrium Dialysis Experiments to Measure Log D

The drug solutions were prepared in PBS adjusted to the desired pH with solutions of HCl or KOH and purged with nitrogen for at least 30 minutes. The concentration of acrivastine in the experiments carried out at pH 7.4 ranged from 108 to 179 μM, while for the experiments at pH 6.0 and pH 8.8 the concentrations ranged from 47 to 78 μM. The concentrations of cetirizine ranged from 418 μM to 1.0 mM, while the concentrations of hydroxyzine ranged from 435 μM to 560 μM.

The liposomal stock suspension was diluted with PBS to give lipid concentrations ranging from 1.16 to 1.48 mg/mL for the experiments with a small molar [lipid]/[ligand] ratio (<4), and lipid concentrations ranging from 10.10 to 33.47 mg/mL for the experiments with a large molar [lipid]/[ligand] ratio (>4). The suspensions were vortexed for 2 minutes, and the pH was checked and adjusted if necessary. The resulting suspensions and solutions were purged with nitrogen for an hour before starting the experiment.

For each compound and each pH value, three dialysis cells were filled with 1 mL of the drug solution in one com-

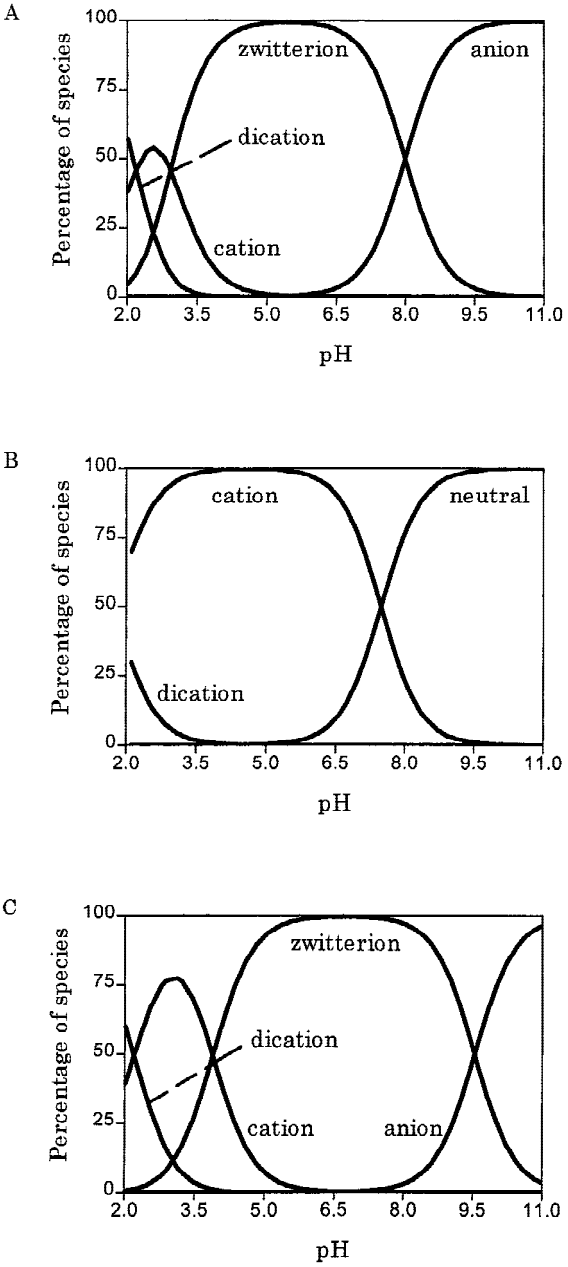

Fig. 2. Ionization plots of cetirizine (A), hydroxyzine (B) and acrivastine (C).

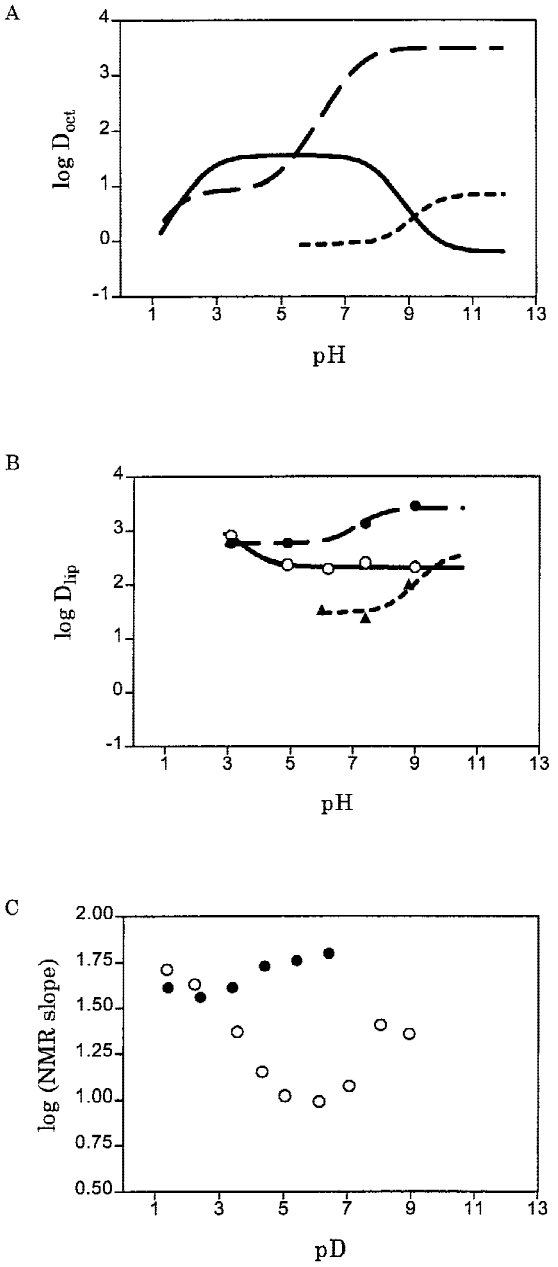

Fig. 3. Distribution profile of cetirizine (——, —○—), hydroxyzine (— —, — —●— —) and acrivastine (– – –, – –▲– –) (A) from potentiometric experiments in *n*-octanol/water (2); (B) from dialysis experiments in PhC liposomes/water; (C) from NMR experiments in PhC liposomes/water. NMR slopes are variations in linewidth of the -CH- signal vs lipid concentration (Table 2 and 4); pH = pD -0.4. Error bars are smaller than symbols.

partment and 1 mL of the liposomal suspension in the other. In addition, one cell was filled with 1 mL of buffer in one compartment and 1 mL of the liposomal suspension in the other. This last cell served as a blank to correct for minute amounts of free lipids that might have crossed the dialysis membrane. The compartments of the dialysis cells were separated by a dialysis membrane previously soaked in PBS for at least 20 minutes.

The cells were rotated at 3 rpm at room temperature. Generally equilibrium was reached after 2 hours, except at pH 9 where equilibrium was reached after 5 hours (data not shown). The cells were emptied and the pH of the liposomal compartments was checked. Drug concentrations were measured by UV spectroscopy at 232 nm for cetirizine and hydroxyzine and 308 nm for acrivastine.

Equation 1 was then used to calculate the distribution coefficient D,

$$D = \frac{V_L(C_L - C_B)}{V_{Lipo} \cdot C_B} + 1 \qquad (1)$$

where C_B and C_L are respectively the molar drug concentrations in the aqueous and liposomal compartment, V_L is the volume of the drug compartment (1 mL) and V_{Lipo} is the volume occupied by the lipid membranes in the liposomal suspension. This latter parameter was calculated from the

lipid concentrations assuming a density of the phosphatidylcholine bilayer of 1.014 g/mL (18).

Calculation of Log P from pK_a and Log D Values

The relationship between log D and log P of the different electrical species of cetirizine, acrivastine and hydroxyzine is described by equation 2 (2), in which P_i represents the partition coefficient of species i and f_i its molar fraction.

$$\log D^{pH} = \log \sum_{i}^{n} (f_i \cdot P_i) \tag{2}$$

By expressing the molar fraction of all electrical species as a function of pK_a and pH, the partition model given in equation 3 can be obtained for cetirizine and acrivastine:

$$\log D^{pH} = \log\left[P^Z \cdot \frac{10^{pK_{a3}-pH}}{X} + P^C \cdot \frac{10^{pK_{a2}+pK_{a3}-2\cdot pH}}{X} + P^A \cdot \frac{1}{X} \right] \tag{3}$$

where

$$X = 1 + 10^{pK_{a3}-pH} + 10^{pK_{a2}+pK_{a3}-2\cdot pH} + 10^{pK_{a1}+pK_{a2}+pK_{a3}-3\cdot pH}$$

For hydroxyzine the equation simplifies to:

$$\log D^{pH} = \log\left[P^C \cdot \frac{10^{pK_{a2}-pH}}{Y} + P^N \cdot \frac{1}{Y} \right] \tag{4}$$

where

$$Y = 1 + 10^{pK_{a2}-pH}$$

With these equations, it is possible to calculate log P values for certain electrical species (log P^N for uncharged species; log P^A for anion; log P^C for cation and log P^Z for zwitterion (19), using (a) a log D value at a pH where the species of unknown log P is present together with a species of known log P; or by (b) combining two log D values (at pH values where the same species exist) expressed as in Eq. 3 and resolving these two equations for the unknown partition coefficients. The molar fractions of the species present at a certain pH can be calculated using the ionization constant(s) and the pH (see Eq. 3 and 4). Filling the calculated log P values in Eq. 4 yields the lipophilicity profile(s). As reported (2), the partitioning of the dicationic forms of cetirizine and hydroxyzine were not taken into account in the log P calculations.

Determination of Log P_{lip} by Potentiometry

Potentiometric titrations of hydroxyzine were performed with the PCA101 apparatus (20) (Sirius Analytical Instruments Ltd, Forrest Row, East Sussex, UK) equipped with an Ag/AgCl double-junction pH electrode (Sirius Analytical Instruments Ltd, Forrest Row, East Sussex, UK), a temperature probe, an overhead stirrer, a precision dispenser and a six-way valve for distribution reagents and titrants (0.5 M HCl, 0.5 M KOH and 0.15 M KCl). Both the weighed sample and the liposomal stock suspension were added manually, whereas the diluent and all other reagents were supplied automatically (21).

At least three different and separate acid titrations were performed with a liposomal suspension. The titrations were carried out under a slow argon flow to avoid CO_2 absorption, at 26.3 ± 0.1°C and in the pH range 10.5 to 3.5, a pH region in which the liposomes are stable. The log P values were estimated from difference Bjerrum plots (20) and refined by a linear least squares procedure (22). The concentrations of hydroxyzine ranged from 0.42 mM to 0.46 mM, the phospholipid concentrations from 0.70 to 3.40 mg/mL and the molar (lipid)/(ligand) ratios from 1.98 to 10.6.

Calcein Release Experiments

After measurement of the blank, which is the fluorescence of the 1000-fold diluted suspension, and after breakdown of the liposomes, we determined the maximal fluorescence, F_{max}, which represents the fluorescence of calcein after its release from the liposomes. Breakdown of the liposomes was provoked by addition of 30 µL 10% Triton X-100 to 3 mL of the 1000-fold diluted liposomal suspensions, followed by sonication during 10 minutes at room temperature.

Suspensions containing cetirizine or hydroxyzine plus calcein-loaded liposomes were prepared with a molar [lipid]/[ligand] ratio of 0.1, because higher [lipid]/[ligand] ratios were shown not to be discriminative. The suspensions were shaken in a warm waterbath at 37°C for 5 hours before their fluorescence (F) was measured. Again a suspension, containing only liposomes but no drug, served as a blank.

The percentage leakage was calculated from equation 5,

$$\% \text{ leakage} = \frac{F - F_B}{F_{max} - F_B} \cdot 100 \tag{5}$$

where F and F_B are the fluorescence of the sample and the blank respectively, while F_{max} is the maximal fluorescence (14,15).

Interactions with Liposomes Measured by NMR

All ^{1}H-NMR spectra were recorded at 200 MHz on a Bruker AC-200 NMR-spectrometer at 298 K. For the theoretical background of this method see (23). Practically for a given solute, the slope obtained by plotting the observed linewidth of an isolated singlet signal at half-peak height ($\Delta\nu_{1/2}$) against the lipid concentration serves as an indicator of the degree of interaction between solute and liposomes. Different slopes (called here NMR slopes) indicate different rotational freedoms of the observed groups in the molecule, thus pointing to different degrees of interaction with phospholipids.

In this study, values of $\Delta\nu_{1/2}$ (in Hz) were obtained for cetirizine in a pD range between 1.5–9, by monitoring the broadening of the benzhydrylic -CH- and the -CH_2-protons close to the carboxylic moiety. In the case of hydroxyzine, only the benzhydrylic -CH- proton was monitored. For acrivastine, the aromatic -CH_3- was selected for measurement, but the very low aqueous solubility of this drug rendered NMR experiments difficult.

Small amounts of liposomes were added to a solution with a constant solute concentration of about 15 mM (see Table III). The lipid/ligand concentration ratios ranged from 0.03 to 0.16, thus avoiding the interference of proton signals from the phospholipids. Broadening was determined for at least 5 different phospholipid concentrations.

RESULTS

Experimental Lipophilicity Data in the Liposomal System

Partition and distribution coefficients in the liposomes/water system as obtained by dialysis experiments at different

Table I. Lipophilicity Parameters in Anisotropic Systems

Distribution	Cetirizine	Hydroxyzine	Acrivastine
log $D_{lip}^{3.1}$	2.91 (± 0.03)[a]	2.76 (± 0.04)[a]	
log $D_{lip}^{4.9}$	2.36 (± 0.21)[a]	2.76 (± 0.09)[a]	
log $D_{lip}^{6.0}$	2.29 (± 0.05)[a]		1.53 (± 0.08)[a]
log $D_{lip}^{7.4}$	2.46 (± 0.04)[a]	3.13 (± 0.11)[a]	1.38 (± 0.06)[a]
log $D_{lip}^{9.0}$	2.32 (± 0.05)[a]	3.46 (± 0.05)[a]	2.01 (± 0.17)[a]
Partitioning[b]			
log P_{lip}^{C}	3.2[c]	2.8[f]	
log P_{lip}^{Z}	2.3[d]		1.5[h]
log P_{lip}^{N}		3.4[g]	
log P_{lip}^{A}	2.3[e]		2.6[i]

[a] Value determined by normal equilibrium dialysis at different lipid/drug ratios (mean ± S.D; n = 3 to 6).
[b] C = cation; Z = zwitterion; N = non-ionized; A = anion.
[c] Calculated value using Eq. 3, log $D^{3.1}$ and log P^{Z}.
[d] Calculated by taking the average of the log D values obtained at pH 4.9 and 6.1, at which cetirizine is in its zwitterionic form (Figure 3).
[e] Equal to log $D^{9.0}$.
[f] Calculated by taking the average of the log $D^{3.1}$ and log $D^{4.9}$.
[g] Calculated value using Eq. 4, log $D^{7.4}$, log $D^{8.9}$ and log P^{C}.
[h] Calculated by taking the average of the log $D^{6.0}$ and log $D^{7.4}$.
[i] Calculated value using Eq. 3, log $D^{9.0}$ and log P^{Z}.

pH values of cetirizine, hydroxyzine, and acrivastine are listed in Table I, while related distribution profiles (plots of log D *vs.* pH) are shown in Fig. 3B. To facilitate comparison with previous results, the ionization constants and relevant lipophilicity parameters of **1, 2,** and **3** in isotropic systems are reported in Table II and their ionization plots and distribution profiles in octanol/water are illustrated in Fig. 2 and Fig. 3A, respectively.

The partitioning of cationic hydroxyzine is almost as high as the partitioning of its neutral form, as deduced by their partition coefficients of 2.8 and 3.4, respectively. Indeed, these values are in excellent agreement with the log P^{C} and log P^{N} values obtained by potentiometry, respectively 2.64 and 3.60. For cetirizine, the zwitterion (log P_{lip}^{Z} = 2.3) is less lipophilic in the liposomes/water system than its cationic species (log P_{lip}^{C} = 3.2) and almost identical to the lipophilicity of the anion (log P_{lip}^{A} = 2.3). Finally, the zwitterionic acrivastine (log P_{lip}^{Z} = 1.5) is more hydrophilic than its anion (log P_{lip}^{A} = 2.6). Its log P^{C} is experimentally inaccessible.

An interesting picture is observed when comparing the distribution plots of cetirizine, hydroxyzine and acrivastine in

Table II. Ionization Constants and Lipophilicity Parameters in Isotropic Systems (log P, log D)

	Cetirizine	Hydroxyzine	Acrivastine
pK_{a1}	2.19[a]	1.75[a]	2.22[a]
pK_{a2}	2.93[a]	7.49[a]	3.89[a]
pK_{a3}	8.00[a]		9.55[a]
log P_{oct}^{C}	1.12[a]	0.93[a]	
log P_{oct}^{Z}	1.55[a]		–0.06[c]
log P_{oct}^{N}		3.50[a]	
log P_{oct}^{A}	–0.19[b]		0.86[c]
log $D_{oct}^{7.4}$	1.5	3.1	–0.06

[a] Value determined by potentiometry (2).
[b] Value obtained by centrifugal partition chromatography (2).
[c] Value obtained in a triphasic system known as Koch's flask (2).

PhC liposomes/water and in *n*-octanol/water. Hydroxyzine and acrivastine, although characterized by different electrical species, have similarly shaped curves (Fig. 3A and 3B). For both compounds, the distribution profile in *n*-octanol/water system was shifted down compared to the profile in the liposomes/water system, and their lipophilicity increased with increasing pH. In particular, the lipophilicity of the neutral form of hydroxyzine in the liposomal system (log P_{lip}^{N} = 3.4) was similar to that evaluated in *n*-octanol/water (log P_{oct}^{N} = 3.50), while the cation gained in lipophilicity when passing from an isotropic (log P_{oct}^{C} = 0.93) to an anisotropic system (log P_{lip}^{C} = 2.8). The same is true for acrivastine on going from the zwitterion to the anion species. Conversely, in the case of cetirizine a decrease in lipophilicity was observed with increasing pH in the anisotropic system, indicating that the zwitterion is less lipophilic than its cationic species (8), while the opposite is true in the *n*-octanol/water system where a bell-shaped curve was obtained (Fig. 3A) (2).

To check for liposome integrity during experimental log D determination, the influence of cetirizine and hydroxyzine on the liposomal membrane stability was studied using calcein-containing liposomes. At pH 7.4, both hydroxyzine and cetirizine did not disrupt the liposomes to any significant extent when the molar lipid/ligand ratio was 0.10. The % leakage after 5 hours were 6.5 ± 5.1% and 3.2 ± 2.9% for hydroxyzine and cetirizine, respectively. These values are similar to the leakage observed in the absence of any drug (data not shown), but much lower than those observed for a typical drug leading to membrane disruption, such as amio-

Table III. Hydroxyzine Linewidth Broadening (sec^{-1}) at Different pH Values[a]

	-CH- group							
pH[b]	Lip/Drug 0.000	Lip/Drug 0.032	Lip/Drug 0.064	Lip/Drug 0.096	Lip/Drug 0.128	Lip/Drug 0.159	Slope	r^2
1.0	1.94	3.64	5.43	6.16	7.24	8.67	40.5 ± 2.6	0.98
2.0	1.84	3.02	3.99	5.34	6.53	7.54	36.2 ± 0.7	0.99
3.0	1.96	3.56	5.27	6.32	7.04	[c]	40.5 ± 3.8	0.97
4.0	2.29	4.09	5.05	6.49	9.77	[c]	54.4 ± 7.2	0.95
5.0	2.41	4.54	6.13	8.15	9.91	11.59	57.4 ± 1.0	0.99
6.0	3.21	5.60	7.80	10.04	11.95	13.00	62.9 ± 3.5	0.98

[a] Mean ± S.D.; S.D < 0.05; n = 3.
[b] pH = pD – 0.4.
[c] Broad signal.

Table IV. Cetirizine Linewidth Broadening (sec^{-1}) at Different pH Values[a]

	-CH_2-group		-CH-group	
pH[b]	Slope	r^2	Slope	r^2
1.0	23.6 ± 0.6	0.99	51.5 ± 1.8	0.99
1.8	72.3 ± 1.4	0.99	42.8 ± 1.4	0.99
3.2	12.5 ± 1.0	0.98	23.6 ± 0.4	0.99
3.9	9.6 ± 1.0	0.98	14.3 ± 0.9	0.98
4.6	6.0 ± 0.3	0.99	10.5 ± 1.1	0.96
5.7	8.0 ± 0.8	0.99	9.9 ± 1.7	0.90
6.7	5.4 ± 0.5	0.98	11.8 ± 2.2	0.88
7.6	8.4 ± 0.6	0.98	25.6 ± 0.99	0.99
8.6	8.4 ± 0.6	0.97	22.8 ± 1.5	0.98

[a] Mean ± S.D.; n = 3.
[b] pH = pD − 0.4.

darone for which a % leakage of 55–65 was found under the same conditions.

T_2 NMR Results and Their Correlation with Lipophilicity

To unravel the complex mechanisms governing the interactions between the compounds under study and biomembranes, changes in NMR relaxation rates were examined (23).

The indicators of the degree of interaction (the NMR slopes) are reported in Table III and IV for each isolated spin system of hydroxyzine and cetirizine as a function of pH. The larger the slope, the higher the degree of interaction between the observed nuclei and phospholipids. The plots of the benzhydrylic CH of both drugs are reported in Fig. 3C.

In the case of hydroxyzine (Table III), all electrical species seem to interact strongly with liposomes. Despite the fact that the pH domain in which the neutral form predomines could not be fully investigated due to solubility problems, the small increment of the slope values at pH > 4.0 indicates that neutral hydroxyzine has a higher affinity for liposomes than its cation.

For cetirizine (Table IV), despite the different location of the two probes (CH and CH_2) in the molecule, the trend towards broadening is similar over the entire pH range. For reason of clarity, only the NMR slope variation for CH is presented in Fig. 3C. The cationic species (1 < pH < 3) interact strongly with the liposomes, anionic cetirizine (pH > 9) binds in an intermediate way, while for zwitterionic cetirizine (4 < pH < 7) binding is minimal.

The similarity between the distribution profiles, obtained either by equilibrium dialysis (Fig. 3B) or by NMR (Fig. 3C) for hydroxyzine and cetirizine, indicates that both methods give the same qualitative information. The plots only differ at pH 8 and 9, where the interactions between cetirizine and the liposomes seem to be stronger than expected based on the profile in Figure 3C.

Finally, the aromatic CH_3 of acrivastine was also studied, but several technical problems (e.g. solubility, features of NMR spectra) made it difficult to measure its interactions with liposomes by NMR. Nevertheless, it appeared that the interaction of zwitterionic acrivastine with liposomes is minute or even nil (plot and data not shown).

DISCUSSION

Our results show that at acidic pH, positively charged cetirizine and hydroxyzine display similar behaviour in liposomes, whereas an opposite trend is observed when neutral pH is approached. This implies that different mechanisms are involved in the partitioning of the two drugs into phospholipids (Table V). In fact, electrostatic bonds between their positive charge and the phosphate groups of liposomes could further reinforce hydrophobic binding between the phenyl groups and the lipidic core. These two cooperative effects could be responsible for the high partition coefficients of the cationic species of the two antihistamines. On the other hand, the different electrical states of the two antihistamines in the pH range 4–7, would play a key role in determining the partitioning properties of their neutral forms. Hydrophobic binding of the two aromatic rings to the bilayer core is the most suitable explanation for the high partitioning of neutral hydroxyzine. In contrast, the low degree of interaction observed for zwitterionic cetirizine suggests that weaker electrostatic surface interactions with polar head groups of phospholipids dominate its partitioning in liposomes.

The flexibility of cetirizine is another important issue that needs to be considered when interpreting the results. In fact, two main classes of conformers, namely folded and ex-

Table V. Modes of Interaction with Liposomes, Deduced from this Study

Electrical species[a]	Cetirizine	Hydroxyzine	Acrivastine
cation	Hydrophobic binding with the lipid core and electrostatic interactions with the phosphate groups	Hydrophobic binding with the lipid core and electrostatic interactions with the phosphate groups	
zwitterion	Predominating electrostatic interactions with the phosphate groups and less important hydrophobic binding with the lipid core		Hydrophobic binding with the lipid core and weak electrostatic interactions with the phosphate groups
neutral form		Hydrophobic binding with the lipid core	
anion	Hydrophobic binding with the lipid core and repulsive electrostatic interactions between the carboxylate and the phosphate groups		Hydrophobic binding with the lipid core and repulsive electrostatic interactions between the carboxylate and the phosphate groups

[a] See Fig. 2 for the corresponding pH ranges.

tended ones with different estimated lipophilicity were identified for zwitterionic cetirizine (2,3). The bell-shaped curve observed in the *n*-octanol/water system could indicate that folded conformers, in which the polarity of the drug is partially masked, dominate the partitioning behaviour of cetirizine in this system. The contrary may be true in the liposomes/water system, in which the partitioning of zwitterionic cetirizine appears to be governed by its extended conformers.

This view is compatible with the results of Kantar *et al.,* who showed that a direct interaction of cetirizine with external plasma membrane components induced an increase in the lipid order of the membrane (24) and thus a decrease in the external membrane fluidity. The surface adsorption of zwitterionic cetirizine may prevent its entry in the liposomal membrane by rendering the formation of lipophilic folded conformers more difficult.

The relatively high log P observed by dialysis experiments for anionic cetirizine implies a high degree of interaction with liposomes, reflecting the balance of repulsive electrostatic interactions between the carboxylate and the phosphate groups, and the hydrophobic interactions with the membrane core. The relevance of hydrophobic interactions for the anion is confirmed by the time (several hours) necessary to reach an equilibrium between the liposomes and the buffer compartment (see experimental section).

Compared to cetirizine, acrivastine shows weaker interactions with liposomes, probably due to its rigid structure where the distance between the ionizable groups may be not optimal to establish strong electrostatic interactions with the liposome surface. The increased distribution coefficient of acrivastine at pH 9 suggests that the anionic form, which has a localized negative charge, may interact with the membrane core by its large lipophilic moiety.

The findings reported in Figure 3A and 3B have pharmacokinetic implications. Thus, hydroxyzine as a lipophilic base shows a fair lipophilicity in the pH region 3–5 rendering duodenal absorption possible, and a very high lipophilicity at pH 7.4 which results in high affinity for lean tissues (its volume of distribution (V_D) is 16.0–30.5 L kg^{-1} (2,25,26)). The same pattern of lipophilicity is seen in liposomes, although here the difference between the cationic and neutral forms is decreased. Cetirizine as an internally compensated zwitterion has a constant and good lipophilicity in the pH-region 4–7, compatible with good GT absorption and restricted tissue distribution, as indeed reported (V_D = 0.4 L kg^{-1}) (2,25,26).

Acrivastine as an internally non-compensated zwitterion has lipophilicity profiles resembling those of hydroxyzine, except that they are shifted to much lower lipophilicities. The prediction here is for a slower GT absorption and a restricted tissue distribution. But while the relations between lipophilicity profiles and pharmacokinetic behaviour are rather well understood for lipophilic bases and internally compensated zwitterions, they remain to be better understood for compounds such as acrivastine.

CONCLUSIONS

The mechanisms governing the interactions between cetirizine and liposomes were studied using various experimental techniques and hydroxyzine and acrivastine as reference compounds.

Our results indicate that each electrical species of cetirizine and hydroxyzine has a particular mode of interaction with liposomes. The neutral form of hydroxyzine mainly interacts via hydrophobic interactions with the bilayer lipid core of the membrane, while its cationic species may interact both via hydrophobic interactions and electrostatic interactions with the negatively charged phosphate groups of the phospholipids. Zwitterionic cetirizine is assumed to interact more by weak electrostatic surface interactions with the polar headgroups than by hydrophobic interactions with the membrane interior. Its extended conformers are well suited to interact with the charged groups of the liposomal surface.

Taken together, these findings are quite different from lipophilicity analysis in the traditional octanol/water system. The fact that cetirizine appears to bind to the membrane surface rather than to enter its core may have pharmacodynamic and pharmacokinetic relevance.

ACKNOWLEDGMENTS

The authors thank Dr. Neuwels, Dr. Rihoux, and Dr. Quéré (UCB Pharma) for their interest and fruitful discussions. BT and PAC acknowledge support by the Swiss National Science Foundation.

REFERENCES

1. K. K. Pandya, R. A. Bangaru, T. P. Gandhi, I. A. Modi, R. I. Modi, and B. K. Chakravarthy. High-performance thin-layer chromatography for the determination of cetirizine in human plasma and its use in pharmacokinetic studies. *J. Pharm. Pharmacol.* **48**:510–513 (1996).
2. A. Pagliara, B. Testa, P. A. Carrupt, P. Jolliet, C. Morin, D. Morin, S. Urien, J. P. Tillement, and J. P. Rihoux. Molecular structure and pharmacokinetic behavior of cetirizine, a zwitterionic antihistamine. *J. Med. Chem.* **41**:853–863 (1998).
3. G. Ermondi, G. Caron, G. Bouchard, G. Plemper van Balen, A. Pagliara, T. Grandi, P. A. Carrupt, R. Fruttero, and B. Testa. Molecular dynamics and NMR exploration of the property space of the zwitterionic antihistamine cetirizine. *Helv. Chim. Acta* (in press).
4. G. V. Betageri and J. A. Rogers. The liposome as a distribution model in QSAR studies. *Int. J. Pharm.* **46**:95–102 (1988).
5. U. Hellwich and R. Schubert. Concentration-dependent binding of the chiral beta-blocker oxprenolol to isoelectric or negatively charged unilamellar vesicles. *Biochem. Pharmacol.* **49**:511–517 (1995).
6. C. Ottiger and H. Wunderli-Allenspach. Ideal partition behaviour of acids and bases in a phosphatidylcholine liposome/buffer equilibrium dialysis system. *Eur. J. Pharm. Sci.* **5**:223–231 (1997).
7. A. Avdeef, K. J. Box, J. E. A. Comer, C. Hibbert, and K. Y. Tam. pH-metric log P. 10. Determination of vesicle membrane-water partition coefficients of ionizable drugs. *Pharm. Res.* **15**:209–215 (1998).
8. A. Pagliara, P. A. Carrupt, G. Caron, P. Gaillard, and B. Testa, Lipophilicity profiles of ampholytes. *Chem. Rev.* **97**:3385–3400 (1997).
9. M. J. Hope, M. B. Bally, G. Webb, and P. R. Cullis. Production of large unilamellar vesicles by a rapid extrusion procedure. Characterization of size distribution, trapped volume and ability to maintain a membrane potential. *Biochim. Biophys. Acta* **812**:55–65 (1985).
10. S. D. Krämer, A. Braun, C. Jakits-Deiser, and H. W. Underli-Allenspach. Towards the predictability of drug-lipid membrane interactions: The pH-dependent affinity of propranolol to phosphatidylinositol containing liposomes. *Pharm. Res.* **15**:739–744 (1998).
11. L. D. Mayer, M. J. Hope, and P. R. Cullis. Vesicles of variable sizes produced by a rapid extrusion procedure. *Biochim. Biophys. Acta* **858**:161–168 (1986).
12. X. R. Qi, Y. Maitani, and T. Nagai. Effect of soybean-derived

sterols on the in vitro stability and the blood circulation of liposomes in mice. *Int. J. Pharm.* **114**:33–41 (1995).
13. X. R. Qi, Y. Maitani, and T. Nagai. Rates of systemic degradation and reticuloendothelial system uptake of calcein in the dipalmitoylphosphatidylcholine liposomes with soybean-derived sterols in mice. *Pharm. Res.* **12**:49–52 (1995).
14. K. Muramatsu, Y. Maitani, Y. Machida, and T. Nagai. Effect of soybean-derived sterol and its glucoside mixtures on the stability of dipamitoylphosphatidylcholine and dipalmitoylphosphatidylcholine/cholesterol liposomes. *Int. J. Pharm.* **107**:1–8 (1994).
15. F. Frézard, C. Santaella, P. Vierling, and J. G. Riess. Permeability and stability in buffer and in human serum of fluorinated phospholipid-based liposomes. *Biochim. Biophys. Acta* **1192**:61–70 (1994).
16. M. Takayama, S. Itoh, T. Nagasaki, and I. Tanimizu, A new enzymatic method for determination of serum choline-containing phospholipids. *Clin. Chim. Acta* **79**:93–98 (1977).
17. P. Trinder. Determination of glucose in blood using glucose oxidase with an alernative oxygen acceptor. *Ann. Clin. Biochem.* **6**:24–27 (1969).
18. S. D. Krämer, C. Jakits-Deiser, and H. Wunderli-Allenspach. Free fatty acids cause pH-dependent changes in drug-lipid membrane interactions around physiological pH. *Pharm. Res.* **14**:827–832 (1997).
19. G. Caron, F. Reymond, P. A. Carrupt, H. H. Girault, and B. Testa. Combined molecular lipophilicity descriptors and their role in understanding intramolecular effects. *Pharm. Sci. Technolog. Today* **2**:327–335 (1999).
20. A. Avdeef. pH-Metric log P. Part 1. Difference plots for determining ion-pair octanol-water partition coefficients of multiprotic substances. *Quant. Struct. -Act. Relat.* **11**:510–517 (1992).
21. G. Caron, P. Gaillard, P. A. Carrupt, and B. Testa. Lipophilicity behavior of model and medicinal compounds containing a sulfide, sulfoxide, or sulfone moiety. *Helv. Chim. Acta* **80**:449–462 (1997).
22. A. Avdeef. pH-Metric log P. II. Refinement of partition coefficients and ionization constants of multiprotic substances. *J. Pharm. Sci.* **82**:183–190 (1993).
23. R. Fruttero, G. Caron, E. Fornatto, D. Boschi, G. Ermondi, A. Gasco, P. A. Carrupt, and B. Testa. Mechanisms of liposomes/water partitioning of (p-methylbenzyl)alkylamines. *Pharm. Res.* **15**:1407–1413 (1998).
24. A. Kantar, J. P. Rihoux, and R. Fiorini. Effect of cetirizine on plasma membrane of human eosinophils, neutrophils and platelets: a dose response study. *Eur. J. Pharm. Sci.* **4**:101–107 (1996).
25. F. E. R. Simons and K. J. Simons. Pharmacokinetic optimization of histamine H_1-receptor antagonist therapy. *Clin. Pharmacokin.* **21**:372–393 (1991).
26. J. P. Tillement and E. Albengres. Peut-on adapter la distribution d'un médicament dans l'organisme aux localisations de ses cibles? L'exemple d'antihistaminiques (anti H_1) et de la cétirizine. *Allergie et Immunologie* **28**:330–332 (1996).