Ion-Pair Partition of Quaternary Ammonium Drugs: The Influence of Counter Ions of Different Lipophilicity, Size, and Flexibility

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Purpose. The ion-pair partition of quaternary ammonium (QA) pharmacons with organic counter ions of different lipophilicity, size, shape and flexibility was studied to elucidate relationships between ion-pair formation and chemical structure.

Methods. The apparent partition coefficient (P') of 4 QAs was measured in octanol/pH 7.4 phosphate buffer system by the shake-flask method as a function of molar excess of ten counter ions (Y), namely: mesylate (MES), acetate (AC), pyruvate (PYRU), nicotinate (NIC), hydrogenfumarate (HFUM), hydrogenmaleate (HMAL), p-toluenesulfonate (PTS), caproate (CPR), deoxycholate (DOC) and prostaglandin E_1 anion (PGE_1).

Results. Based on 118 of highly precise logP' values (SD< 0.05), the intrinsic lipophilicity (without external counter ions) and the ion-pair partition of QAs (with different counter ions) were characterized. Linear correlation was found between the logP' of ion-pairs and the size of the counter ions described by the solvent accessible surface area (SASA). The lipophilicity increasing effect of the counter ions were quantified and the following order was established: DOC ~ PGE_1 ≫ CPR ~ PTS ≫ NIC ~ HMAL ≫ PYRU ~ AC ~ MES ~ HFUM. Analyzing the lipophilicity/molar ratio (QA:Y) profile, the differences in the ion-pair formation were shown and attributed to the differences in the flexibility/rigidity and size both of QA and Y.

Conclusions. Since the largest (in average, 300 ×) lipophilicity enhancement was found by the influence of DOC and PGE_1 and considerable (on average 40 ×) increase was observed by CPR and PTS, it was concluded that bile acids and prostaglandin anions may play a significant role in the ion-pair transport of quaternary ammonium drugs and caproic acid and p-toluenesulfonic acid may be useful salt forming agents to improve the pharmacokinetics of hydrophilic drugs.

KEY WORDS: ion-pairing; partition coefficients; quaternary ammonium drugs, anionic counter ions.

INTRODUCTION

The paradigm of pH-partition theory, widely accepted for the absorption/distribution mechanism of drugs, can be well applied for neutral molecules and weak electrolytes (1). This principle postulates that nonionized, lipophilic drugs are able to penetrate across the membrane barriers. However, the pH-partition theory fails to elucidate the absorption behavior of highly ionized drugs (like strong bases, peptides) or permanent ions (like quaternary ammonium salts: QA). Considering the alternatives of absorption the active transport has been proved to be used by sugars, amino acids and some small drug molecules (e.g., L-Dopa, 5-fluorouracil etc.), the carrier facilitated diffusion is applied by some biomolecules and certain vitamins. Another way of transport is the ion-pair partition of charged drugs, where the hydrophilic pharmacon (cation or anion) forms a less polar, more lipophilic species with appropriate ions of opposite charge. This concept has been hotly debated in the pharmaceutical literature. Many of pro (2–6), and contra (7–9) arguments have been registered.

Recently, Quintanar-Guerrero *et al.* (10) reviewed the application of the ion-pair concept in term of peptide's pharmacokinetics. In their article, the physicochemistry and the analytical applications of ion-pair formation, and the pharmacological aspects have been summarized. Another review was focused on the hydrophobic ion-pairing (HIP), a method for altering the solubility of biopolymers (peptide, protein or polynucleotide) without chemical modification (11). It has been shown that hydrophobic ion-pair complexes display enhanced lipophilicity, and thus, enhanced ability to cross the biological barriers. However, other authors found that HIP has not led to an increase in tissue uptake or membrane permeability of lutein hormone releasing hormone (LHRH) analogues (12).

The research activity of Neubert *et al.* made large contribution to the acceptance of ion-pair concept in pharmacy confirming, that ion-pairing with an appropriate counter ion is a good tool to improve the bioavailability of a hydrophilic drug (13–17). However, the question, which counter ion can be considered ideal or at least suitable, has remained unanswered. The conclusion can be drawn from the above cited papers is the ion-pair partition is a possible transport mechanism, but a better understanding of the correlation between the physicochemical properties of ion-pairs and their interactions with membranes is necessary (10). The first step to this purpose should be a thorough and systematic investigation of ion-pair partition itself.

In this paper, we report an extensive study on the ion-pair partition of orally active quaternary ammonium drugs. Four QAs have been selected: propantheline (**1**), trantheline (**2**) (spasmolytics), homidium (**3**) (anthelmintic) and neostigmine (**4**) (cholinergic), in which the quaternary N atom is present in different chemical surroundings (aliphatic, alicyclic, aromatic and aniline type). The octanol/water partition coefficients have been determined using the traditional shake-flask method. Lipophilicity was characterized by the experimental logP values both without external counter ions (intrinsic lipophilicity) and in the presence of ten different (endogenous and exogenous) organic counter ions. The ion-pairing counter ions used in this study vary significantly in their hydrophobicity, size, shape, flexibility, etc.

The aims of our investigations were: (i) to reveal the relationship between the ion-pair partition and the chemical structure both for the pharmacon and the counter ion; (ii) to show the role of noncolumbic molecular interactions throughout the ion-pair formation; and (iii) to quantify the effect of counter ions in enhancing the lipophilicity, which characterization may be applied in drug research.

EXPERIMENTAL

Materials

Propantheline bromide was granted by CHINOIN Pharm. Works (Budapest, Hungary) and trantheline bromide was supplied by EGIS Pharmaceuticals (Budapest, Hungary). Homidium bromide (=ethidium bromide) and neostigmine bromide

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were purchased from Sigma-Aldrich Chemie (Germany). The QA drugs were used without further purification. Materials used as counter ions were also purchased from Sigma-Aldrich either acid (methanesulfonic, maleic, fumaric, p-toluenesulfonic, nicotinic acids) or in salt form (sodium acetate, caproate, pyruvate and deoxycholate). Prostaglandin E_1 was generously supplied by Chinoin Pharm. Works. All other reagents were of analytical grade. The n-octanol was of HPLC grade (Sigma-Aldrich).

Methods

The shake-flask method was applied for the logP measurements as was suggested by Dearden and Bresnen (18) and validated by us (19).

Sörensen buffer pH 7.4 (potassium dihydrogenphosphate and disodium hydrogenphosphate dihydrate, each at 0.067 M) was used generally as aqueous phase in the partitioning measurement. The ionic strength was kept constant (I = 0.27M). In the case of bivalent (maleic and fumaric) acids—taking into consideration their pK_a values (pK_{a1}: 2.78, pK_{a2}: 4.05 and pK_{a1}: 1.92, pK_{a2}: 6.23, resply)—Britton-Robinson buffer pH 3.5 (acetic, phosphoric and boric acids, each at 0.04 M and 0.2 M sodium hydroxide) was applied to assure the dominance of monoanions ($HMAL^-$: 67.9%, $HFUM^-$: 99.8%) in the solution. The aqueous and octanol phases were mutually saturated.

The QA salts, depending on their molar absorptivity, were dissolved in the aqueous phase, in 5×10^{-5} M (homidium), 1×10^{-4} M (propantheline, trantheline) 1×10^{-3} M (neostigmine) concentration. Six different QA:Y molar ratios were used, from 1:1 to 1:50. Attention had been paid to keep the concentration of the surfactant anions below the CMC in order to avoid the micelle formation. If literature values were not available (e.g., for CPR) or they were controversial (e.g., for DOC) the CMC was experimentally measured by dynamic surface tension method. No micelle formation was observed at caproic acid in 10^{-2} M–10^{-4} M range (used for our partition experiments), while CMC of DOC in octanol saturated pH 7.4 Sörensen buffer was found above 7×10^{-3} M. So maximum 5 mM of DOC was applied at the highest, 1:50 QA:Y molar ratio. Therefor the concentration of neostigmine was reduced to 1×10^{-4} M in partition experiments with DOC counter ion and 5 cm cuvette was used in spectrophotometric measurements.

The pH of the aqueous phase was controlled having added the counter ions to the system and when decrease in the pH was observed (approx. 0.5 pH caused by the strong sulfonic acids) it was adjusted back to 7.4 value by 0.2 M NaOH.

The phase volumes (V_w/V_o) varied from 10:1 (ml) to 5:50 (ml), exceptionally 3.5:70 (ml) depending on the expected logP′ value of the ion-pairs. The phases were equilibrated at 25.0°C in a shaking thermostat (Lauda M2OS, Germany). After centrifugation, the concentration measurements were carried out in the aqueous phase by UV spectroscopy (Hewlett-Packard 8452A, diode array spectrometer) at the highest λ_{max} of each QA. The apparent partition coefficients were calculated according to Eq. 1.

$$P' = \frac{(A_o - A_1)}{A_1}\frac{V_w}{V_o} \qquad (1)$$

where A_o and A_1 represent the absorbance of QA in the aqueous phase before and after partitioning, respectively. Each log P′ value is an average of minimum four, generally six parallel measurements. The standard deviations (SD) of the results were generally less than 0.05 log unit and are indicated in the tables.

RESULTS AND DISCUSSION

The formation of an ion-pair in aqueous solution and its partition into an immiscible organic solvent (like octanol) are described by Eq. 2 in Scheme 1. Here the cation is a quaternary ammonium pharmacon (QA^+) which interacts with a counter ion (Y^-) forming a less polar ion-pair. For this equilibrium the K_{ip}, formation (or stability) constant of ion-pair is relevant (Eq. 3). The distinct and thermodynamically stable species (QA^+Y^-) formed partitions between the aqueous and organic phases which equilibrium can be characterized by the (true) partition coefficient (P) of the ion-pair (Eq. 4). The related other equilibrium constants (apparent partition coefficient of the ion-pair, P′, and that of the cation, P'_{QA}; extraction constant, K_{ex}) and their relationships are summarized in Scheme 1 by Eqs. 5–9.

The concept above supposes the existence of ion-pairs even in aqueous solutions which may emerge when the ions involved are relatively hydrophobic. Systems of this type, called "water structure enforced" ion-pairing was introduced by Diamond in early sixties (20) and have been considered in this paper. It has also been established in the formation of such type of ion-pairs, out of the electrostatic forces other interactions, e.g., hydrophobic and polar ones, play also significant role.

Four quaternary ammonium drugs (structures, see in Fig. 1) have been selected as model compounds for some specific reasons. First, the partitioning ability of these molecules being

$$QA^+_w + Y^-_w \rightleftharpoons (QA^+Y^-)_w \rightleftharpoons (QA^+Y^-)_o \qquad (2)$$

$$K_{ip} = \frac{[QA^+Y^-]_w}{[QA^+]_w [Y^-]_w} \qquad (3)$$

$$P = \frac{[QA^+Y^-]_o}{[QA^+Y^-]_w} \qquad (4)$$

$$P' = \frac{[QA^+Y^-]_o}{[QA^+]_w + [QA^+Y^-]_w} \qquad (5)$$

$$P'_{QA+} = \frac{[QA^+Y^-]_o}{[QA^+]_w} \qquad (6)$$

$$K_{ex} = \frac{[QA^+Y^-]_o}{[QA^+]_w [Y^-]_w} \qquad (7)$$

$$\log K_{ex} = \log P' - \log[Y^-]_w \qquad (8)$$

$$\log P = \log K_{ex} - \log K_{ip} \qquad (9)$$

Scheme 1. Equilibrium constants related to ion-pair formation and partition.

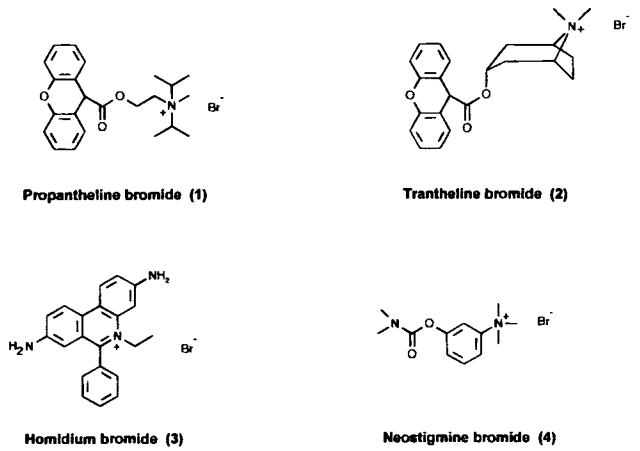

Fig. 1. Structures of model compounds: quaternary ammonium drugs.

orally active compounds is interesting. Secondly, as permanent cations they provide model for protonated amines without the pH of the medium is to be considered. Finally, they represent four different types of QAs as concerns the chemical environment of the quaternary N atom (aliphatic: comp. **1**, alicyclic: comp. **2**, aromatic: comp. **3** and aniline: comp. **4**), providing sufficient variety for conclusion on the influence of the structure on the ion-pair formation.

The counter ions used in this study, together with some characteristic molecular and physico-chemical parameters, are shown in Fig. 2. They cover a wide range of lipophilicity (from MES to DOC) and differ considerably in size, shape, flexibility (CPR vs TOS or PGE_1 vs DOC, etc.). Most of them are human endogenous (e.g., AC, FUM, MAL, PYRU, DOC, PG, etc.), others appear as anions in drugs (MES, TOS, etc.). The carboxylic acids are completely ionized at tissue pH (7.4), while sulfonic acids can be considered as permanent anions at any physiological pH values including the gastric pH (1.5) as well.

Lipophilicity of QA Model Compounds

The logP value of QA drugs in bromide salt form as used in medicine (without external counter ions), was determined by shake-flask technique in order to describe the "intrinsic" lipophilicity of these molecules. Data in Table I show that compds **1–3** have low, whereas neostigmine has extremely low lipophilicity. For this latter molecule no partition could be measured from the aqueous phase into octanol using shake-flask method at 1:20 (w/o) phase ratio, which findings indicate a logP value less than −3.

The mass balance was controlled representatively by propantheline, measuring the concentration in both phases. We also investigated the composition of the partitioning ion-pair at compd. **1**. Stoichiometric amount of bromide ion was found in the octanol phase relative to propantheline cation, which result has proved no competitive exchange between Br^- and the HPO_4^{2-} ions of the buffer.

The effect of the excess of own counter ion (bromide in our case) on the apparent partition coefficient of compd. **1** was investigated in the presence of KBr at 1:2; 1:10; 1:50 (QA:Y) molar ratios and the found logP′ values are: −1.00 (0.03), −0.72(0.01), −0.22(0.03), resply. It is to be noted that a similar small increase in the lipophilicity of charged drugs may be expected with other physiological inorganic counter ions, as was reported by Jafvert *et al.* (21).

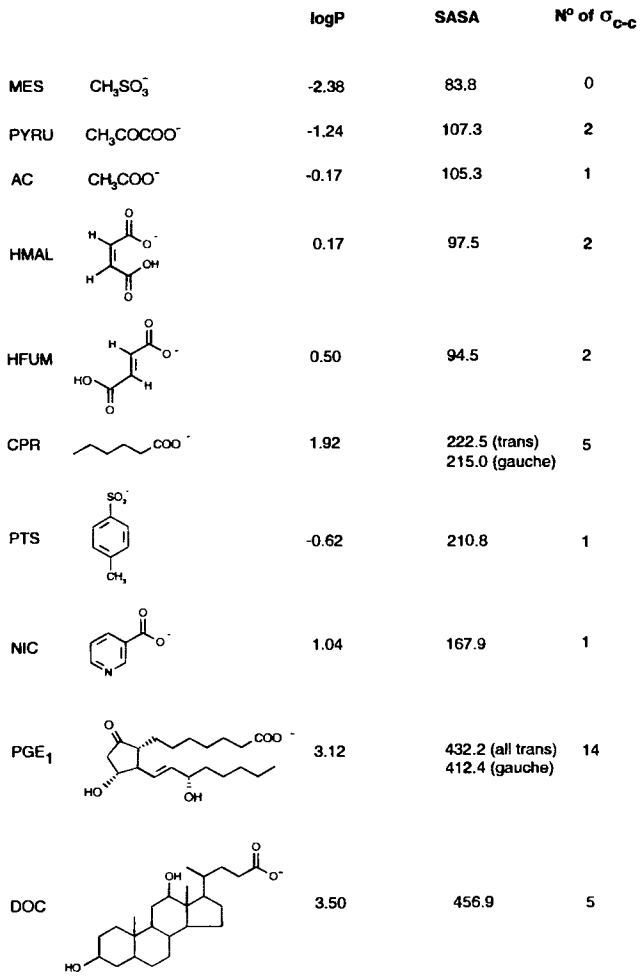

Fig. 2. Structures and molecular properties of ion-pairing counter ions. (log P: partition coefficient of non-ionized form; SASA [A^2]: solvent accessible surface area for conformers minimized by MM+ method [22]; flexibility expressed by the number of rotating C-C σ bonds in chain) abbreviations of counter ions used in the text: MES (mesylate), PYRU (pyruvate), AC (acetate), HMAL (hydrogenmaleate), HFUM (hydrogen-fumarate), CPR (caproate), PTS (p-toluenesulfonate), NIC (nicotinate), PGE_1 (prostaglandin E_1 anion), DOC (deoxycholate).

Effect of Counter Ions on the Lipophilicity of QAs

Next, the influence of externally added counter ions was studied. The measured logP′ values of QA^+Y^- ion-pairs are collected in Table II. The logP′ changes caused by the increasing

Table I. Lipophilicity of QA Model Compounds

Compound	log P (±SD)
1. propantheline bromide	−1.07 (0.05)
2. trantheline bromide	−1.45 (0.02)
3. homidium bromide	−1.10 (0.01)
4. neostigmine bromide	<−3

Table II. The Logarithm of Apparent Partition Coefficients of QA Ion-Pairs

Compound	Counter ion	log P′ QA^+: Y^- molar ratio 1:1	1:2	1:5	1:10	1:25	1:50
Propantheline	MES	−1.32 (0.02)	−1.31 (0.01)	−1.30 (0.01)	−1.21 (0.04)		−0.79 (0.01)
	AC	−1.32 (0.01)	−1.33 (0.01)	−1.33 (0.01)	−1.20 (0.01)		−0.79 (0.01)
	HMAL*	−1.17 (0.04)	−0.93 (0.01)	−0.54 (0.02)	−0.17 (0.09)		0.18 (0.03)
	CPR	−0.89 (0.01)	−0.35 (0.01)	−0.14 (0.02)	0.08 (0.01)	0.40 (0.02)	0.64 (0.01)
	PTS	−0.67 (0.12)	−0.32 (0.01)	0.05 (0.02)			
	DOC	−0.92 (0.03)	0.86 (0.02)	1.15 (0.03)	1.14 (0.05)		1.14 (0.10)
	PGE_1	−1.01 (0.04)		0.80 (0.02)	1.01 (0.01)		1.23 (0.02)
Trantheline	MES	−1.35 (0.01)		−1.26 (0.03)	−1.25 (0.04)		−0.87 (0.03)
	CPR	−0.91 (0.02)		−0.25 (0.02)	−0.01 (0.01)	0.36 (0.03)	0.65 (0.01)
	DOC	−0.88 (0.02)		1.08 (0.03)	1.17 (0.12)	1.59 (0.05)	2.14 (0.05)
	PGE_1	−0.78 (0.01)		0.72 (0.01)	0.94 (0.01)		1.35 (0.03)
Homidium	MES	−1.12 (0.01)		−1.10 (0.01)	−1.07 (0.02)		−0.80 (0.02)
	AC	−1.11 (0.03)		−1.02 (0.01)	−0.96 (0.01)	−0.87 (0.02)	−0.67 (0.01)
	HFUM*	−1.11 (0.01)		−1.08 (0.01)	−1.11 (0.01)		−1.05 (0.02)
	HMAL*	−0.80 (0.01)		−0.47 (0.01)	−0.30 (0.01)		−0.02 (0.01)
	PYRU	−1.06 (0.01)		−1.05 (0.03)	−0.96 (0.01)		−0.67 (0.01)
	NIC	−0.84 (0.01)		−0.72 (0.01)	−0.59 (0.02)	−0.37 (0.02)	−0.14 (0.01)
	CPR	−0.61 (0.02)		−0.18 (0.01)	−0.01 (0.01)	0.25 (0.01)	0.48 (0.01)
	PTS	−0.78 (0.02)		−0.32 (0.02)	−0.06 (0.01)		0.53 (0.01)
	DOC	−0.52 (0.01)		0.11 (0.01)	0.64 (0.01)	1.97 (0.01)	2.18 (0.01)
	PGE_1	−0.49 (0.01)		0.15 (0.01)	0.58 (0.01)		1.33 (0.01)
Neostigmine	MES	<−3		<−3	<−3		<−3
	CPR	<−3		−2.2 (0.10)	−2.0 (0.08)	−1.72 (0.05)	−1.38 (0.01)
	DOC	<−3		−2.3 (0.10)	−1.65 (0.04)	−1.05 (0.03)	−0.70 (0.06)
	PGE_1	−2.2 (0.02)		−1.53 (0.04)	−0.95 (0.03)	−0.25 (0.02)	

* pH:3.5

excess of counter ions can be generally described with saturation type curves as it is representatively shown in Fig. 3. Capabilities of the Y anions for increasing the lipophilicity are markedly different. HFUM has no effect and that of MES and AC are almost negligible. CPR and PTS counter ions caused similar and considerable increase in logP′. On average, a three times enhancement in lipophilicity was observed at 1:1 molar ratio, and a 40 times increase at 50 molar excess of CPR and PTS counter ions. The largest change in the partition properties was produced by DOC and PGE_1 ions, where maximum, appr. 2000 times (DOC) and 300 times (PGE_1) increases were reached with the homidium cation. The outstanding ion-pairing abilities of DOC and two other bile acids for increasing lipophilicity have also been found in our RP HPLC investigations to be published elsewhere.

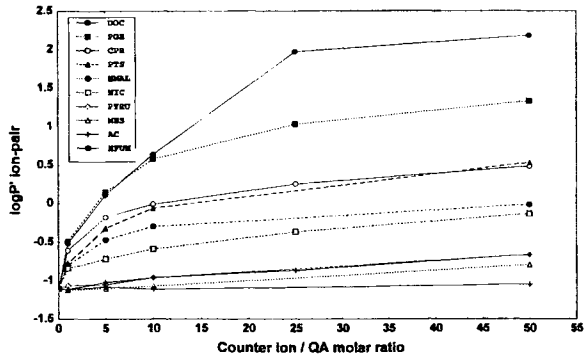

Fig. 3. Influence of counter ions on the lipophilicity of homidium.

The analysis of the enhancement in lipophilicity suggests that the size of the counter ion may be a determining factor. Linear correlation has been found between the logP′ values of homidium ion-pairs (measured at 1:50 molar ratio) and the solvent (water) accessible surface area (SASA) of the counter ion calculated by the Generalized Born/Surface Area continuum model implemented in the MacroModel 6.0 program (22).

$$\log P'_{HOM\text{-}Y} = 0.007\ SASA - 1.311 \qquad (10)$$

$$r = 0.972\ s = 0.184\ n = 8$$

The correlation analysis does not include the data by the bivalent acids.

Figure 4 exhibits the partition behavior of propantheline in the presence of the bivalent maleic acid at two different pH values. At pH 7.4 MAL^{2-} ion is dominant, thus if ion-pairing should occur at all, a species of negative charge can be formed and the log P′ of ion-pair is decreased relatively to the intrinsic lipophilicity of **1**. However, at pH 3.5 due to the predominance of $HMAL^-$ ion and the formation of a neutral ion-pair, the expected log P′ enhancement can be observed.

Based on 118 of highly precise experimental logP′ values, the lipophilicity increasing effect of the counter ions could be quantified and the following order has been established:

$$DOC \sim PGE_1 \gg CPR \sim PTS \gg NIC \sim HMAL \gg PYRU \sim AC \sim MES \sim HFUM$$

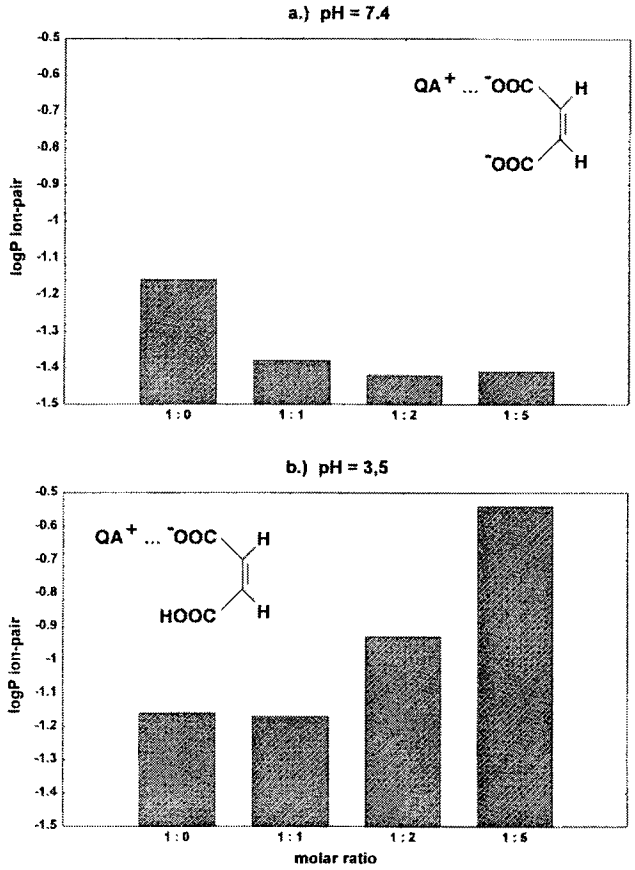

Fig. 4. Differences in ion-pairing and partition of propantheline with maleic acid at pH 7.4 (a) and 3.5 (b) values.

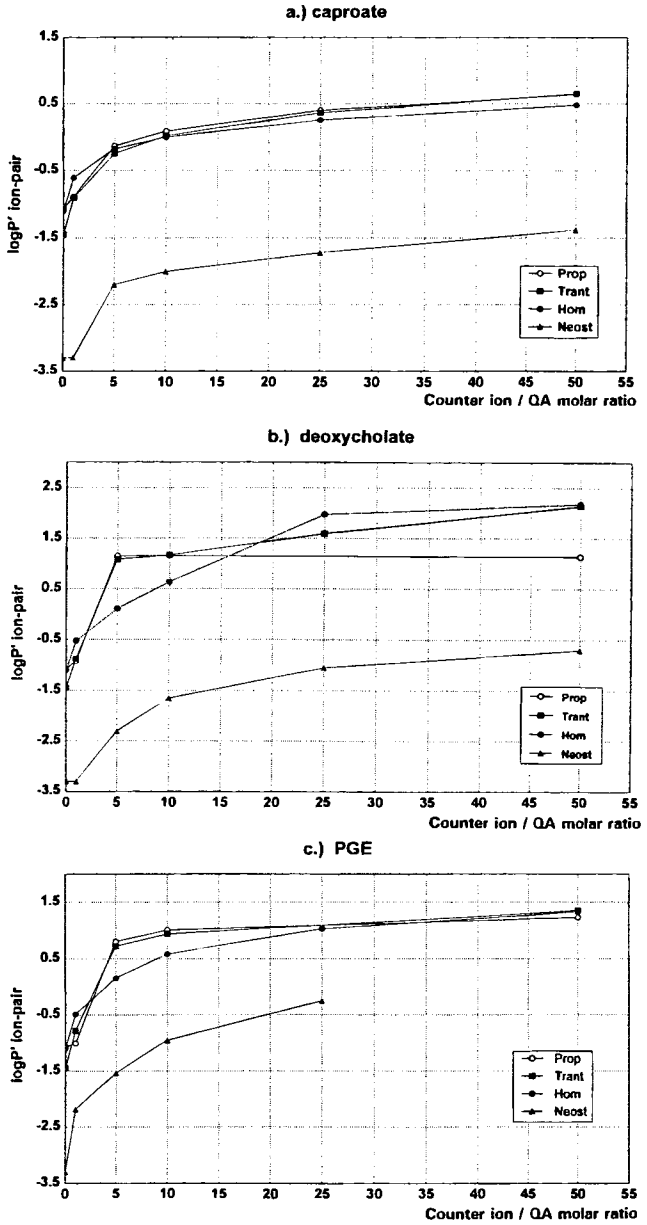

Fig. 5. Influence of caproate (a), deoxycholate (b) and prostaglandin E_1 ion (c) on the lipophilicity of different QA drugs.

These results significantly extend the information regarding the lipophilizing effect of counter ions that have been used so far (3,23). The formerly investigated inorganic ions can fit into this order preceding and succeeding mesylate ($Br^- > NO_3^- \gg Cl^- >$ (MES) $\gg SO_4^{2-}$).

Relationships Between Ion-Pair Formation and Chemical Structure of Interacting Species

Analysis of the lipophilicity / molar ratio profiles considering the same counter ion with different QAs (Fig. 5), provides useful information on the relationships between the ion-pair formation and the chemical structure. Similarity of the curves (there is only a shift along the y axis) means similar ion-pair formation capabilities. Dissimilarities of these curves (different shape, slope, etc.) reveal differences in the ion-pair formation. This is demonstrated in Fig. 5. There is no significant difference in the ion-pair formation of QAs **1-4** with the relatively small caproate ion (SASA: 222A^2) (Fig. 5a). Neither the chemical environment of the quaternary N atom nor the flexibility of QAs do influence the ion-pairing with this counter ion. Dissimilarities can be observed, however, with DOC (Fig. 5b), and also with PGE_1 (Fig. 5c). DOC being a large, rigid and hydrophobic counter ion (SASA: 457 A^2) interacts reluctantly with homidium which is also a large, lipophilic, rigid molecule containing quaternary N atom in aromatic moiety. Though the hydrophobic interactions between the two skeletons may contribute to the stability of the ion-pair, the suitable orientation of these moieties is more difficult due to the rigid structures of the ions. At homidium, the completion of the ion-pair formation and the maximum lipophilicity have been reached only at higher molar excess of counter ion than in other case with more flexible cations, as propantheline and trantheline. These latter two ions show identical ion-pairing behavior, while neostigmine exhibits similarity to homidium. These results support the statement about the importance of the shape, size, conformational flexibility in the ion-pair formation.

CONCLUSIONS

Based on investigations for ion-pair partition of 4 QA drugs with 10 counter ions, the following conclusions have been drawn.

The lipophilicity enhancement ability of organic counter ions is mainly determined by their size. Linear correlations exists between log P′ of ion-pair and by the SASA-expressed size of the counter ions.

The largest effect—increase in the partition coefficient by a factor of 300 in average—was caused by DOC and PGE_1, consequently these species can be considered as feasible endogenous counter ions for ion-pair absorption of hydrophilic cations (protonated amines or QAs). Caproic acid and p-toluenesulfonic acid produced a 40x increase in the lipophilicity of QAs in average, so these agents may be used as salt forming acids to improve the pharmacokinetics of potential drugs. The small, hydrophilic counter ions (MES, AC, PYRU) are useful only in charge neutralization when the pharmacon has a sufficiently high intrinsic lipophilicity for penetration.

It has also been established that the size and flexibility of both ions (QA^+ and Y^-) seem to be essential in the ion-pair formation. However, no significant effect has been found due to the type (e.g., aliphatic or aromatic) of the quaternary N atom.

Our results support the possible role of endogenous lipophilic counter ions (e.g., bile acids, prostaglandins, etc.) in the GI absorption of QA drugs. Neostigmine bromide, an orally active cholinergic molecule, has a log P value less than −3, which postulates no absorption in this form. However, the presence of 50 molar excess of DOC can produce more than 200 times increase in lipophilicity (logP′: −0.70). We note, that exact evidence for ion-pair transport can be provided by *in vivo* pharmacological experiments which are in progress.

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