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Report

The Liposome as a Model Membrane in Correlations of Partitioning with α-Adrenoceptor Agonist Activities[1]

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The apparent partition coefficients of a group of imidazoline α-adrenoceptor agonists in liposome/buffer systems (K'_m) and in the *n*-octanol/buffer system (P') have been compared in quantitative structure–activity relationships (QSAR) employing biological activities and receptor binding affinities. A parabolic relationship between log K'_m and log P' was found, and log K'_m was greater than log P' for all liposome compositions. In liposomes, log K'_m decreased in the order, negatively charged > neutral > positively charged. Overall, hyper- and hypotensive activities of these drugs correlated better with log K'_m than with log P'; however, poor correlations were obtained between partition coefficients and *in vitro* binding affinities. Linear correlations of log K'_m with hypotensive activities were obtained with negatively charged liposomes, whereas correlations with hypertensive activities were obtained using positively charged liposomes. Multiple regressions of biological activities with binding affinities showed positive correlations with hypotensive but not hypertensive activities with or without the inclusion of log K'_m or log P'. Thus, the liposome represents a more selective model membrane system than a bulk oil phase for predicting the biological activities of imidazoline α-adrenoceptor agonists.

KEY WORDS: partitioning; α-adrenoceptor agonists; liposome; correlation analysis; quantitative structure–activity relationships (QSAR).

INTRODUCTION

Imidazoline derivatives have been introduced generally as partial agonists for α-adrenergic receptors, and activities of sympathomimetics have been investigated in terms of molecular properties based on structure–activity relationships (1,2). Quantitative relationships between structure and hypotensive activity of clondine-like imidazolines and between biological activity and binding affinity of α-adrenoceptor agonists and antagonists have been established (3,4). Affinity for receptors and the ability to reach the site of action are usually thought to depend on various factors including molecular structure, pK_a, and lipid solubility of drug (2,5,6). Although lipophilicity is a property that can be used to predict the membrane transport of solutes from linear relationships, parabolic or bilinear relationships have often been found when highly lipophilic compounds are included, e.g., log $P > 2$ (7).

The partitioning of solutes into bilayer membranes has been argued by some investigators to occur by a fundamentally different mechanism than partitioning into a bulk oil phase, e.g., *n*-octanol, using evidence of the thermodynamics of the process (8–12), NMR and IR (13,14), the influence of membrane structure in liposomes of different compositions (15–17), the existence or absence of surface charges (15,18), and quantitative structure–activity relationships (QSAR) studies (18). Furthermore, the liposome adequately predicts membrane transport, pharmacokinetic behavior, and biological activities of certain classes of drugs (19–23). Hence, studies of the liposome/buffer partitioning behavior have been extended to the imidazoline class of compounds, and the relevance of this membrane model in making predictions of biological activities has been evaluated.

MATERIALS AND METHODS

Materials. The structures of the α-adrenoceptor agonists used in this study are depicted in Fig. 1. All compounds, as their hydrochloride salts, were obtained as follows: tramazoline (Boehringer Ingelheim Canada Ltd.), tiamenidine (Hoechst Canada Inc.), lofexidine (A. Nattermann & Cie. GMBH), cirazoline (Synthelabo Recherche, LERS, France), naphazoline, tetrahydrozoline (tetryzoline), clonidine, xylometazoline, and oxymetazoline (Sigma Chemical Co.). L-α-Dimyristoylphosphatidylcholine (DMPC), cholesterol (CHOL), dicetylphosphate (DCP), phosphatidylserine (PS) from bovine brain, and stearylamine (STA) (Sigma Chemical Co.) were used as received. All other solvents and chemicals were reagent grade and water was glass-distilled.

Partition Coefficient Determinations. Partition coefficients of the α-adrenoceptor agonists in *n*-octanol/pH 7.4 phosphate buffer (P') at 37°C have been previously reported

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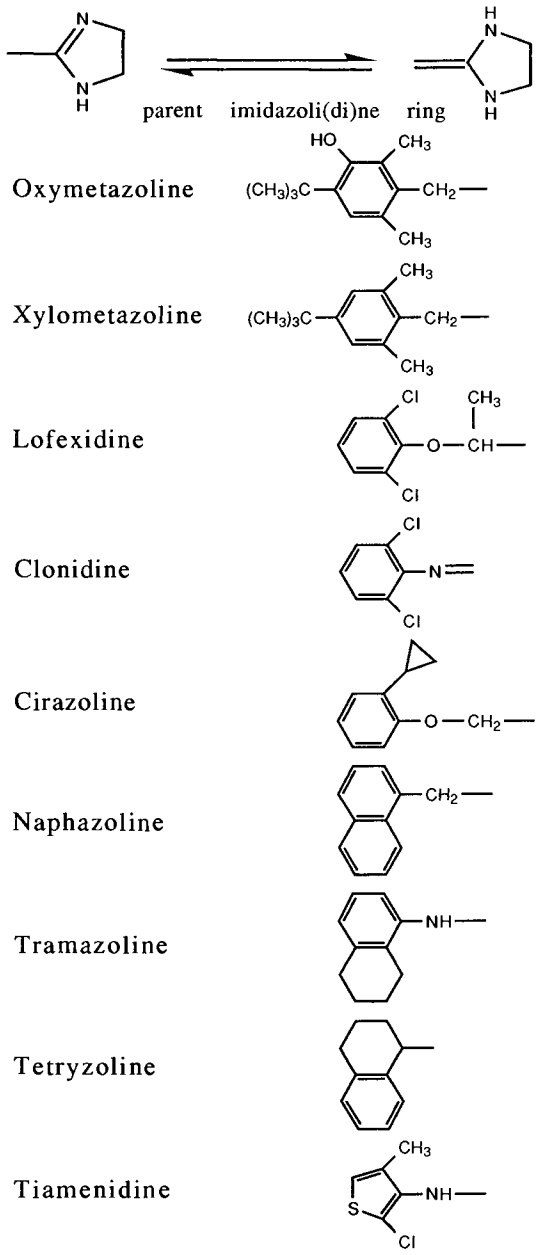

Fig. 1. Chemical structures of α-adrenoceptor agonists.

(3). Apparent equilibrium partition coefficients in liposomes (K'_m) were determined by a method previously described (18). Briefly, dried films of phospholipids obtained by rotary evaporation of chloroform solutions in 25-ml round-bottom flasks were hydrated and hand-dispersed in aqueous, pH 7.4, phosphate buffer solution (μ = 0.15) containing 1 m*M* drug and then vortex-mixed for 5 min to form multilamellar liposomes (MLVs) at a concentration of 10 mg/ml. After equilibration of the MLVs at 37°C for 5 hr in a shaking water bath (Dubnoff metabolic shaking incubator, Precision Scientific Co.), samples were centrifuged (Beckman Model L8-55 Ultracentrifuge; 143,000*g*, 30 min, 37°C) and the supernatants were analyzed by UV spectrophotometry. Mass balance calculations were employed to obtain concentrations of drug in liposomes, and calculations of K'_m were made as before (18). Averages of duplicate determinations have been reported. Repeated analyses of stock solutions confirmed the stabilities of the drugs under the experimental conditions.

RESULTS AND DISCUSSION

The partition coefficients of nine imidazoline derivatives in liposomes of various compositions determined experimentally and in the *n*-octanol/buffer system obtained from the literature (3) are compared in Table I. In all cases, values of log K'_m were found to be greater than log P', which may be attributed to attractive electrostatic interactions between the cationic imidazoline and anionic regions of the liposomes, which are absent in the *n*-octanol phase. Also, an increasing order in the values of log K'_m was not the same as that of log P'. Instead, parabolic relationships were obtained as shown in Fig. 2 (excluding tiamenidine as discussed later). This behavior suggests that partitioning in liposomes is dominated by hydrophilic-group interactions up to $K'_m \simeq 1$ but beyond this the influence of the hydrophobic effect, i.e., hydrocarbon chain interactions, becomes important but also diminishes the hydrophilic influence. Nonlinear regression analysis revealed that log K'_m values correlated reasonably well with log P' values, although negatively charged liposomes containing DCP or PS yielded somewhat better correlations than neutral or positively charged liposomes containing STA [Eqs. (1)–(4), Table II]. The positively charged liposomes yielded decreases in log K'_m except for lofexidine and clonidine, which may be attributed to the electronegative chlorine atoms in their structures (see Fig. 1).

The observed rank order of log K'_m in liposomes, being negatively charged > neutral > positively charged, is consistent with that reported by Schweikert and Roth (15) and indicates that an electrical charge on the membrane surface plays an important role in interactions of these drugs with phospholipid membranes. Furthermore, among different liposome systems, linear relationships of partition coefficients have been established in accordance with the Collander

Table I. Apparent Partition Coefficients of α-Adrenoceptor Agonists in the *n*-Octanol/Buffer (log P') and Liposome/Buffer (log K'_m) Systems at 37°C

Drug	log P'[a]	log K'_m[b] (1)	(2)	(3)	(4)
Oxymetazoline	−0.32	1.94	2.50	2.96	1.16
Xylometazoline	0.40	1.94	2.40	2.80	1.30
Cirazoline	0.53	1.72	2.23	2.65	1.15
Tramazoline	−0.62	1.48	2.17	2.59	0.77
Naphazoline	−0.52	1.34	2.12	2.45	0.70
Lofexidine	0.73	1.24	1.76	2.20	1.20
Clonidine	0.85	1.15	1.61	2.01	1.17
Tiamenidine	−0.17	1.02	1.53	1.94	0.61
Tetryzoline	−0.90	0.95	1.43	1.80	0.55

[a] From Ref. 3.

[b] The numbers in parentheses represent different liposome compositions: (1) DMPC; (2) DMPC/CHOL/DCP, 7:1:2 mol ratio; (3) DMPC/PS, 3.5:1 mol ratio (the average MW of brain PS was taken as 798 ± 4 on the basis of various fatty acid compositions given in Ref. 29); (4) DMPC/STA, 3:1 mol ratio. The maximum standard deviation (SD) was ±5%, although in most cases it was <±2%.

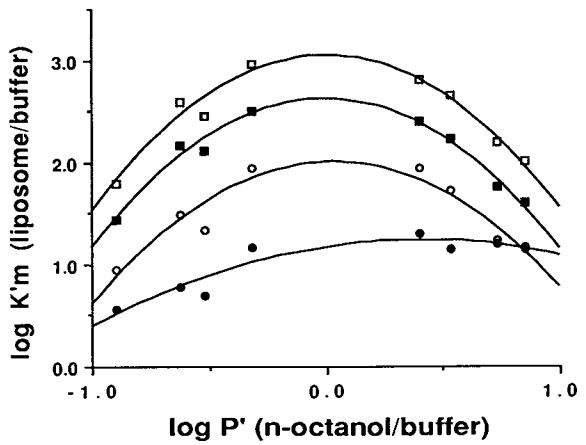

Fig. 2. Parabolic relationships of apparent partition coefficients of α-adrenoceptor agonists between *n*-octanol/buffer (log P') and liposome/buffer (log K'_m) systems: (○) DMPC; (■)DMPC/CHOL/DCP, 7:1:2 mol ratio; (□) DMPC/PS, 3.5:1 mol ratio; (●) DMPC/STA, 3:1 mol ratio.

Table III. Hypertensive (pC_{60}) and Hypotensive (pC_{25}) Activities, Binding Affinities (pD) for α_1- and α_2-Adrenoceptors, pK_a's, and van der Waals Volumes (V_w) of α-Adrenoceptor Agonists

Drug	pC_{60}[a]	pC_{25}[a]	pD-α_1[a]	pD-α_2[a]	log V_w[b]	pK_a[c]
Oxymetazoline	2.24	—	0.52	2.30	2.22	—
Xylometazoline	1.12	0.26	0.24	1.64	2.20	10.20
Cirazoline	2.36	—	0.05	1.23	2.09	—
Tramazoline	1.80	0.55	−0.04	1.80	2.10	10.66
Naphazoline	1.83	0.95	0.39	2.32	2.08	10.35
Lofexidine	1.99	2.09	0.18	2.60	2.10	9.28
Clonidine	1.78	2.04	−0.08	2.51	2.05	8.05
Tiamenidine	1.20	0.69	−0.69	2.04	2.03	9.30
Tetryzoline	0.90	−0.16	−0.20	1.52	2.08	10.51

[a] For details, see Refs. 3 and 26: C_{60} and C_{25} are the doses (micromoles per kilogram) required to evaluate mean arterial pressure to 60 mm Hg and to cause a 25% decrease in mean arterial pressure in rats, respectively; *in vitro* binding affinities are expressed as $pD = -\log IC_{50}$, where IC_{50} is the micromolar concentration inhibiting the specific [^{3}H]prazosin binding (α_1) or [^{3}H]clonidine binding (α_2) to rat brain membranes by 50%.

[b] Molecular van der Waals volumes (V_w) were calculated by summation of the fragmental volumes (see Ref. 30).

[c] From Refs. 2 and 31.

equation. For instance, good correlations were obtained between negatively charged and neutral liposome systems. On the other hand, the use of positively charged liposomes yielded a poor correlation with either of the above systems [Eqs. (5)–(10), Table II]. This suggests that the diffusion environment and partitioning properties of neutral and negatively charged liposomal membranes are similar but different from those of positively charged liposomes.

Correlations of log P' and log K'_m with biological activities and binding affinities of α-adrenoceptor agonists, as defined in Table III, are described in Table IV. Biological activities correlated significantly ($P < 0.01$) with the log K'_m of certain liposome compositions, e.g., hypertensive activities with positively charged liposomes [Eq. (14)] and hypotensive activities with negatively charged liposomes [Eqs. (17) and (18)]. However, both hyper- and hypotensive activities not only correlated poorly with log P' [Eqs. (15) and (20)], but also correlated less significantly with neutral DMPC liposomes [Eqs. (11) and (16)]. These results suggest that an electronic effect is also a very important parameter for the transport of α-adrenoceptor agonists across cell membranes. Thus, the liposome partitioning system could be considered a more selective distribution model for these α-adrenoceptor agonists than the *n*-octanol/buffer system, possibly indicating the significance of the greater biological resemblance of liposomes to biological membranes. On the other hand, no significant correlations were obtained with *in vitro* binding affinities to the receptor and log P' or log K'_m, suggesting that this behavior is more complex than can be predicted from considerations of lipophilicity or simple phospholipid bilayer interactions alone.

A consideration of the structural requirements of the imidazolines may provide a better understanding of the application of regression correlations with respect to their activities as α-adrenoceptor agonists. Ruffolo (24) has examined segments of the structures of these compounds as three essential portions: the intact imidazoline ring, the carbon or nitrogen bridge, and the aromatic ring. As shown in Fig. 1, all the drugs possess these requirements except tiamenidine and tetryzoline. Tiamenidine, which has a heteroaromatic ring, was excluded from regressions involving hypotensive activities or binding affinities, as well as in the plot of log

Table II. Correlations Between Apparent Partition Coefficients of α-Adrenoceptor Agonists

	Equation[a]	R/df/S/P[b]
Eq. (1)	$\log K'_m(1) = 2.01 + 0.09\log P' - 1.14(\log P')^2$	0.935/7/0.024/0.006
Eq. (2)	$\log K'_m(2) = 2.63 - 0.01\log P' - 1.46(\log P')^2$	0.984/7/0.007/0.0002
Eq. (3)	$\log K'_m(3) = 3.05 + 0.02\log P' - 1.51(\log P')^2$	0.972/7/0.012/0.0007
Eq. (4)	$\log K'_m(4) = 1.17 + 0.35\log P' - 0.44(\log P')^2$	0.940/7/0.013/0.005
Eq. (5)	$\log K'_m(1) = -0.38 + 0.91\log K'_m(2)$	0.960/8/0.013/0.0001
Eq. (6)	$\log K'_m(1) = -0.70 + 0.89\log K'_m(3)$	0.970/8/0.010/0.0001
Eq. (7)	$\log K'_m(1) = 0.61 + 0.84\log K'_m(4)$	0.659/8/0.091/0.053
Eq. (8)	$\log K'_m(2) = -0.33 + 0.97\log K'_m(3)$	0.996/8/0.001/0.0001
Eq. (9)	$\log K'_m(2) = 1.31 + 0.69\log K'_m(4)$	0.510/8/0.133/0.160
Eq. (10)	$\log K'_m(3) = 1.65 + 0.76\log K'_m(4)$	0.546/8/0.134/0.128

[a] For definitions of numbers in parentheses within equations, see Table I, footnote *b*.

[b] R = correlation coefficient; df = degree of freedom; S = mean square error; P = probability.

Table IV. Univariant Regression Correlations Between Apparent Partition Coefficients and Pharmacological Parameters of α-Adrenoceptor Agonists

	Equation	x variable[a]	R/df/S/P[b]
Eq. (11)	$pC_{60} = -5.35 + 9.54x - 3.04x^2$	log K'_m(1)	0.750/8/0.151/0.083
Eq. (12)	$pC_{60} = -7.03 + 8.56x - 2.03x^2$	log K'_m(2)	0.649/8/0.200/0.194
Eq. (13)	$pC_{60} = -7.74 + 7.49x - 1.44x^2$	log K'_m(3)	0.636/8/0.206/0.211
Eq. (14)	$pC_{60} = -6.60 + 18.80x - 9.77x^2$	log K'_m(4)	0.922/8/0.052/0.003
Eq. (15)	$pC_{60} = 1.83 + 0.28x - 0.37x^2$	log P'	0.398/8/0.290/0.595
Eq. (16)	$pC_{25} = 4.50 - 2.32x$	log K'_m(1)	0.851/4/0.263/0.067
Eq. (17)	$pC_{25} = 6.33 - 2.56x$	log K'_m(2)	0.972/4/0.052/0.005
Eq. (18)	$pC_{25} = 7.46 - 2.61x$	log K'_m(3)	0.962/4/0.072/0.009
Eq. (19)	$pC_{25} = 0.26 + 0.90x$	log K'_m(4)	0.288/4/0.875/0.637
Eq. (20)	$pC_{25} = 1.04 + 0.80x$	log P'	0.655/4/0.545/0.230
Eq. (21)	$pD\text{-}\alpha_1 = -0.50 + 0.43x$	log K'_m(1)	0.643/7/0.041/0.085
Eq. (22)	$pD\text{-}\alpha_1 = -0.81 + 0.46x$	log K'_m(2)	0.726/7/0.033/0.041
Eq. (23)	$pD\text{-}\alpha_1 = -0.94 + 0.44x$	log K'_m(3)	0.716/7/0.034/0.045
Eq. (24)	$pD\text{-}\alpha_1 = -0.17 + 0.30x$	log K'_m(4)	0.346/7/0.062/0.400
Eq. (25)	$pD\text{-}\alpha_1 = 0.45 + 0.01x - 0.77x^2$	log P'	0.812/7/0.029/0.067
Eq. (26)	$pD\text{-}\alpha_2 = 3.50 - 0.93x$	log K'_m(1)	0.598/6/0.199/0.156
Eq. (27)	$pD\text{-}\alpha_2 = 3.93 - 0.89x$	log K'_m(2)	0.562/6/0.212/0.188
Eq. (28)	$pD\text{-}\alpha_2 = 4.19 - 0.85x$	log K'_m(3)	0.551/6/0.216/0.199
Eq. (29)	$pD\text{-}\alpha_2 = 2.21 - 0.14x$	log K'_m(4)	0.063/6/0.309/0.891
Eq. (30)	$pD\text{-}\alpha_2 = 2.05 - 0.05x$	log P'	0.064/6/0.309/0.891

[a] For definitions of numbers in parentheses, see Table I, footnote *b*.
[b] Defined in Table II, footnote *b*.

K'_m versus log P' (Fig. 2), because of its large deviation from the correlations. Yet it was included in the correlations of hypertensive activity with either log P' or log K'_m (Table IV). In the case of tetryzoline, attachment of the methylene bridge to the ortho position of the phenyl ring via a propyl chain results in a rigidity to conformational change required for receptor binding. According to Avbelj and Hadzi (25), the ortho steric factors and the conformational entropy as a function of the ring interplanar torsional angle are the important parameters in binding of clonidine-like imidazolines to the receptor. Thus, deviation of tetryzoline from the linear relationships between hypotensive activity or α_2-adrenoceptor binding affinity and either log P' or log K'_m may be accounted for if the arguments of Avbelj and Hadzi (25) are applied. On the other hand, such conformational steric requirements are of minor importance with regard to the hypertensive activities of tiamenidine and tetryzoline. Timmermans *et al.* (26–28) have explained the hypotensive and hypertensive activities as central medullary mediated and peripherally (vascular) induced activities, respectively. Thus, based on this differentiation, it is further suggested that the central medullary mediated α-adrenoceptor activity, which is mainly α_2-receptor elicited, has a stereoselectivity requirement.

Possible correlations from multiple regressions of the biological activities, binding affinities, log K'_m, log P', and van der Waals volume as a steric factor were examined and the data are presented in Table V. Interestingly, hypertensive activities (pC_{60}) correlated poorly with binding affinities and van der Waals volumes [Eq. (31)], whereas a strong

Table V. Multiple Regressions of Biological Activities with Binding Affinities, van der Waals Volumes, and Apparent Partition Coefficients of α-Adrenoceptor Agonists

	Equation[a]	R/df/S/P[b]
Eq. (31)	$pC_{60} = 9.11 + 1.21pD\text{-}\alpha_1 - 3.55\log V_w$	0.614/8/0.215/0.242
Eq. (32)	$pC_{60} = 19.54 + 1.03pD\text{-}\alpha_1 - 9.37\log V_w + 1.28\log K'_m(1)$	0.776/8/0.165/0.171
Eq. (33)	$pC_{60} = 14.23 + 0.84pD\text{-}\alpha_1 - 6.87\log V_w + 0.96\log K'_m(2)$	0.737/8/0.189/0.235
Eq. (34)	$pC_{60} = 15.93 + 0.83pD\text{-}\alpha_1 - 8.09\log V_w + 1.15\log K'_m(3)$	0.791/8/0.155/0.148
Eq. (35)	$pC_{60} = 10.94 + 1.04pD\text{-}\alpha_1 - 4.74\log V_w + 0.72\log K'_m(4)$	0.698/8/0.212/0.303
Eq. (36)	$pC_{60} = 8.43 + 1.14pD\text{-}\alpha_1 - 3.22\log V_w + 0.22\log P'$	0.673/8/0.227/0.350
Eq. (37)	$pC_{25} = -6.69 + 2.00pD\text{-}\alpha_2 + 1.66\log V_w$	0.961/6/0.084/0.005
Eq. (38)	$pC_{25} = -9.39 + 2.03pD\text{-}\alpha_2 + 3.01\log V_w - 0.24\log K'_m(1)$	0.962/6/0.110/0.034
Eq. (39)	$pC_{25} = -11.78 + 2.07pD\text{-}\alpha_2 + 4.46\log V_w - 0.49\log K'_m(2)$	0.969/6/0.089/0.024
Eq. (40)	$pC_{25} = -10.84 + 2.07pD\text{-}\alpha_2 + 4.02\log V_w - 0.40\log K'_m(3)$	0.966/6/0.098/0.028
Eq. (41)	$pC_{25} = 6.17 + 1.35pD\text{-}\alpha_2 - 4.39\log V_w + 1.26\log K'_m(4)$	0.989/6/0.031/0.005
Eq. (42)	$pC_{25} = -0.59 + 1.48pD\text{-}\alpha_2 - 0.74\log V_w + 0.39\log P'$	0.982/6/0.054/0.011

[a] For definitions of numbers in parentheses within equations, see Table I, footnote *b*.
[b] Defined in Tabe II, footnote *b*.

correlation was obtained with hypotensive activities (pC_{25}) [Eq. (37)]. Inclusion of log K'_m or log P' parameters slightly improved the correlations with pC_{60} [Eqs. (32)–(36)], although these were of low statistical significance ($P > 0.05$). Also, inclusion of log K'_m or log P' only slightly improved the correlations with pC_{25} [Eqs. (38)–(42)], although these were of high statistical significance ($P < 0.05$).

These results demonstrate that multiple regressions involving binding affinities and van der Waals volumes can reasonably correlate the hypotensive activities, the lipophilic interactions between solute and membrane, and electrostatic interactions at negatively charged sites, but not hypertensive activities, which have greater dependence on membrane structure, composition, and surface charge. Thus, only positively charged liposomes showed a strong correlation with pC_{60} in univariant regression analysis (Table IV). It is, therefore, concluded that log P' alone is completely unsuitable for QSAR studies of the imidazolines examined, whereas log K'_m, using liposomes having either positive or negative charges, can be used to predict the hypertensive or hypotensive activities, respectively.

Previous studies in our laboratory have led to an understanding that differences in membrane interactions of compounds, within a given series, are due to differences in membrane component interactions with the solute molecules, resulting in changes in the partitioning environment because of membrane reorganization, and not just lipophilicity (19). Timmermans and van Zwieten (1) have proposed a hypothetical working model of the electron exchanges between drug and receptor based on the mechanism of interaction of the centrally acting hypotensive imidazolines at the central α-adrenoceptor. Thus, in addition to an electrostatic interaction between a positively charged nitrogen atom of the imidazoline ring and a negatively charged site at the receptor, it is argued that the aromatic portion of the imidazolines interacts with the receptor by means of electron exchange under conditions which are consistent with the geometrical fitness of the donor to the acceptor and the minimum energy requirement. The results obtained in the present study (Tables IV and V) support this working model and it is becoming clear that a structured model membrane system, typified by the liposome system, is superior to the bulk oil/water system in many QSAR applications.

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REFERENCES

1. P. B. M. W. M. Timmermans and P. A. van Zwieten. *J. Med. Chem.* 20:1636–1644 (1977).
2. H. S. Boudier, J. de Boer, G. Smeets, E. J. Lien, and J. van Rossum. *Life Sci.* 17:377–386 (1976).
3. P. B. M. W. M. Timmermans, A. de Jonge, M. J. M. C. Thoolen, B. Wilffert, H. Batink, and P. A. van Zwieten. *J. Med. Chem.* 27:495–503 (1984).
4. A. de Jonge, P. B. M. W. M. Timmermans, and P. A. van Zwieten. *Quant. Struct.-Act. Relat.* 3:138–143 (1984).
5. A. Carpy, J. M. Leger, G. Leclerc, N. Decker, B. Rouot, and C. G. Wermuth. *Mol. Pharmacol.* 21:400–408 (1982).
6. J. C. Dearden. *Environ. Health Perspect.* 61:203–228 (1985).
7. E. J. Lien and P. H. Wang. *J. Pharm. Sci.* 69:648–650 (1980).
8. J. A. Rogers and S. S. Davis. *Biochim. Biophys. Acta* 598:392–404 (1980).
9. G. V. Betageri and J. A. Rogers. *Int. J. Pharm.* 36:165–173 (1987).
10. S. A. Simon, W. L. Stone, and P. Busto-Latorre. *Biochim. Biophys. Acta* 468:378–388 (1977).
11. N. H. Anderson, S. S. Davis, M. James, and I. Kojima. *J. Pharm. Sci.* 72:443–448 (1983).
12. A. M. S. Ahmed, F. H. Farah, and I. W. Kellaway. *Pharm. Res.* 2:119–124 (1985).
13. L. R. de Young and K. A. Dill. *Biochemistry* 27:5281–5289 (1988).
14. D. Hadzi and J. Kidric. *Spectrosc. Biol. Mol., Proc. Eur. Conf., 1st, Reims,* 1985, pp. 277–282.
15. L. Schweikert and H. J. Roth. *Arch. Pharm. (Weinheim)* 321:721–724 (1988).
16. H. G. L. Coster and D. R. Laver. *Biochim. Biophys. Acta* 861:406–412 (1986).
17. J. F. Nagle and M. C. Wiener. *Biochim. Biophys. Acta* 942:1–10 (1988).
18. G. V. Betageri and J. A. Rogers. *Int. J. Pharm.* 46:95–102 (1988).
19. G. V. Betageri and J. A. Rogers. *Pharm. Res.* 6:399–403 (1989).
20. R. Neubert, W. Fuerst, and B. Wurschi. *Pharmazie* 42:102–103 (1987).
21. H. Miyoshi, H. Maeda, N. Tokutake, and T. Fujita. *Bull. Chem. Soc. Jpn.* 60:4357–4362 (1987).
22. J. de Gier. *Advances in Inflammation Research, Vol. 1,* Raven Press, New York, 1979, pp. 7–17.
23. R. E. Brown and B. J. Brown. *QSAR Des. Bioact. Compd.,* Prous, Barcelona, 1984, pp. 13–24.
24. R. R. Ruffolo, Jr. *Neurotransm. Recept. 1* (Adrenoceptors Catecholamine Action, Pt. B.), 1983, pp. 1–50.
25. F. Avbelj and D. Hadzi. *Mol. Pharmacol.* 27:466–470 (1985).
26. P. B. M. W. M. Timmermans, A. de Jonge, J. C. A. van Meel, E. Lam, F. P. Slothorst-Grisdijk, and P. A. van Zwieten. *J. Med. Chem.* 24:502–507 (1981).
27. P. B. M. W. M. Timmermans and P. A. van Zwieten. *J. Med. Chem.* 25:1389–1401 (1982).
28. P. B. M. W. M. Timmermans and P. A. van Zwieten. *Eur. J. Pharmacol.* 45:229–236 (1977).
29. D. Papahadjopoulos and N. Miller. *Biochim. Biophys. Acta* 135:624–638 (1967).
30. A. Bondi. *J. Phys. Chem.* 68:441–451 (1964).
31. R. J. Summers, B. Jarrot, and W. J. Louis. *Eur. J. Pharmacol.* 66:233–241 (1980).