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PARTITION AND DISTRIBUTION COEFFICIENTS OF SOLUTES AND DRUGS IN BRUSH BORDER MEMBRANE VESICLES

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Abstract—Partition and distribution coefficients (log P, log D) into rat small intestinal brush border membrane (BBM) were measured for a variety of ionizable and non-ionizable drugs and solutes using a novel technique. The log P values were compared with those determined with model solvents, octanol and propylene glycol dipelargonate (PGDP). Non-ionizable solutes with log P values up to 3.0 showed that octanol was a better model for partition into the BBM than PGDP. With one exception, BBM partition coefficients of greater than 3 were not observed, even for solutes with log P values in model solvents that were greater than 5. Liposomes prepared from BBM lipids, or synthetic lipid mixtures of similar composition to BBM, demonstrated similar trends in partition coefficients to the intact BBM. Two cationic drugs, Atenolol® and Xamoterol® were investigated for partition into BBM lipid liposomes. An apparent enhancement of log D with respect to octanol was attributed to a "surfactant-like" orientation in the membrane and an interaction of the ionized drug with anionic phospholipid head groups. The anionic drug Proxicromil® shows the expected decrease in log D with increasing pH, at low NaCl concentrations. Changes in electrophoretic mobility of liposomes after incorporation of Proxicromil into them were consistent with the negative charge of the ionized drug being at the membrane surface. It was concluded that Proxicromil also associates with membranes in a "surfactant-like" orientation and that increased extraction with increasing NaCl concentrations is a result of ionic strength effects. Partition of solutes into BBM vesicles is more complex than into organic solvents and probably represents an important step in overall intestinal permeation of solutes.

The design of pharmaceutical agents is currently hampered by an inability to predict reliably the efficiency of their intestinal absorption. Currently, semi-empirical rules are combined with *in vivo* studies in experimental animals and man. Solute partition/distribution coefficients in model solvents are a useful predictor of intestinal absorption but are not sufficiently reliable to replace *in vivo* studies [1–3]. Further investigation of fundamental physicochemical mechanisms of solute absorption is required in order to enable prediction of drug absorption to be made. The rate at which a drug passes from the intestinal lumen to the blood depends on a number of factors: (i) the concentration of drug in the gut lumen, which may be limited by its solubility, (ii) the rate at which the drug diffuses up to the absorptive epithelium through the unstirred aqueous layer, the mucus lining the gut wall and within the cell layer, (iii) the extent to which the drug partitions into both apical and basal cell membranes, and (iv) the diffusivity of the drug in the apical and basal cell membranes. In addition, the gut wall is thought to be a "leaky" epithelium [4] where there is extensive paracellular absorption arising from the solvent drag effect on drugs in lumenal solution.

One important element in this process is the extent of partitioning into the relevant cell membranes and the extent to which the chemical and physical properties of the drug influence this process. This paper addresses two specific issues concerned with partitioning into cell membranes. The first is how the molecular structure of neutral solutes affects the partition coefficient between aqueous solution and brush border membrane vesicles (BBMVs‡) and whether organic solvent partition coefficients serve as useful models for this. At a practical level, we have investigated which of two model solvents available for correlation studies [octagonal and propylene glycol dipelargonate (PGDP)] is the better model for the BBMV partitioning [5]. The second issue is the role of drug ionization in partitioning, in particular the extent to which ionic forms of drugs are able to partition into membranes and whether this is influenced by the nature and concentration of the counter-ion [6].

To address the first question, we report in this paper the BBMV partition coefficients (log P[BBMV]) of 26 solutes and the relationships between log P[BBMV] and partition coefficients measured in octanol, log P[OCT] and PGDP, log P[PGDP]. We have also studied the partitioning into BBMVs of the highly ionized drug Proxicromil® and the influence of factors such as pH, ionic strength and counter-ion concentration, in order to test

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‡ Abbreviations: BBM, brush border membrane; BBMV, BBM vesicle; PGDP, propylene glycol dipelargonate; HEPES, 4-hydroxyethylpiperazine ethanesulphonic acid; DMPC, dimyristoylphosphatidylcholine.

the claims that the enhancement in Proxicromil absorption arising from changes in counter-ion concentration is a result of ion-pair extraction into the membrane. Finally, we discuss the relationship between the extent of partitioning into a membrane at equilibrium with the permeability of that membrane, particularly in view of the greater availability of membrane partition coefficient data.

MATERIALS AND METHODS

Materials. PGDP (Emerest 2388) was obtained from Emery Industries Inc.; octanol, methanol, acetonitrile, chloroform, buffers, salts, mannitol, solutes and drugs were from the Aldrich Chemical Co. (Poole, U.K.) or the ICI Pharmaceuticals compound collection. Lipids and biochemicals were from the Sigma Chemical Co. (Poole, U.K.). Proxicromil was a gift from Dr Payling of Fisons Pharmaceuticals (Loughborough, U.K.).

BBMVs, lipids and liposomes. Intestinal BBMV were prepared from Sprague–Dawley strain rats (male/female, 240–450 g, given free access to food and water until killed) as described previously [1]. BBM lipids were extracted with chloroform/methanol (2:1, v/v) as described previously [7] and multi-lamellar liposomes were prepared as in Ref. 8 with a BBM lipid extract or a mixture of commercially available lipids [phosphatidylethanolamine (egg yolk), 100 mg; phosphatidylcholine (fresh egg yolk), 50 mg; phosphatidylserine (bovine brain), 35 mg; cholesterol (porcine liver), 117 mg; linoleic acid (synthetic free acid), 11 mg; stearic acid (synthetic free acid), 11 mg; sphingomyelin (bovine brain), 17.5 mg; all purchased from Sigma and greater than 98% pure]. Liposomes with differing charges were prepared from egg lecithin/cholesterol (1:1) mixtures with no addition for neutral liposomes, one mass of stearylamine for positive or one mass of phosphatidic acid for negative liposomes. Multilamellar liposomes were prepared as above and sonicated with 5 × 30 sec bursts at 30 sec intervals using an MSE Soniprep 150 sonicator.

HPLC analysis of solute concentrations. Solutes were selected and a UV absorbance scan obtained with a Beckman DU-7 spectrophotometer to determine an optimum detection wavelength. Two solutes with very low water solubility, but significant fluorescence emission, were analysed for optimal excitation and emission wavelengths with a Perkin–

Table 1. Solvents and detection conditions for separation and assay of solutes by HPLC

Solute	Solvent mixture (%)			Detection wavelength (nm)
	Acetonitrile	Methanol	H_2O	
2-Methylanthracene	70	—	30	260
4-Chloro-1-naphthol	60	—	40	235
Naphthalene	50	—	50	275
Phenanthrene	65	—	35	252
Fluoranthrene	70	—	30	235
Methylbenzoate	40	—	60	260
Naphthylsulphonamide	30	—	70	230
Naphthylamide	30	—	70	220
Benzylaldehyde	35	—	65	245
Phenylurea	30	—	70	235
Aniline (0.5% PSA)	20	—	80	252
Methylphenylsulphoxide	20	—	80	240
Hydrochlorthiazide	20	—	80	270
Methylphenylsulphone	20	—	80	265
Benzenesulphonamide	20	—	80	252
Benzamide	30	—	70	223
Proxicromil	60	—	40	260
3-(2-Napthoxy)-propylmethylsulphoxide	—	65	35	230
Anisole	—	55	45	225
Triphenylphosphine	—	50	50	223
Methylnaphthylsulphone	—	60	40	260
Benzonitrile	—	40	60	260
Acetophenone	—	50	50	230
Nitrobenzene	—	50	50	225
Dimethylphenylsulphonamide	—	47	53	225
Atenolol (0.2% SLS)	—	60	40	225
Xamoterol (0.2% PSA)	—	50	50	225
Phenol	—	43	57	230
Phenyl benzamide	—	50	50	260
1,2,5,6-Dibenzanthracene	—	80	20	ex-292 em-405
Chrysene	—	80	20	ex-370 em-410

All the solvent mixtures contained 0.1% trifluoroacetic acid. Some mixtures contained sodium lauryl sulphate (SLS) or pentane sulphonic acid (PSA) as ion-pair reagents to aid separation. Detection was by UV absorption or fluorescence spectrophotometry.

Elmer model 204 scanning fluorimeter. Solutes were dissolved in water and injected onto a Spherisorb S50S1 reverse phase column fitted to a LDC HPLC system (Waters, Croxley, U.K.) fitted with a UV detector or a Kontron spectrofluorimeter set at maximum sensitivity. An optimum water/solvent mixture (methanol or acetonitrile) was determined to allow resolution of each solute, the marker benzoic acid (which was protonated by addition of trifluoroacetic acid to the eluent mixture) and any membrane debris (Table 1).

Partitioning of solutes. Solute partitioning into BBMV or liposomes was determined (see Ref. 1) by incubating solute and benzoic acid (aqueous phase marker), dissolved in 100 μL 20 mM HEPES (4-hydroxyethylpiperazine ethanesulphonic acid) -KOH buffer (pH 7.4) containing 1 mM $MgSO_4$ and 100 mM mannitol, with 50 μL of BBM or liposome suspension for 20 min at room temperature in polytetrafluoroethylene-capped titanium micro-reaction/centrifuge tubes designed to fit the Beckman Airfuge (Beckman Instruments Ltd, High Wycombe, U.K.). The mixture was centrifuged for 15 min at 150,000 *g* and the supernatant removed and injected onto the HPLC analysis system. The difference between the solute concentration, relative to the water phase marker, before and after partitioning, was used to calculate a partition or distribution (ionizable solutes) coefficient.

The pH profile of the distribution coefficient for Proxicromil was determined using a series of buffers, namely: for pH 3.0–5.5, 20 mM citrate-NaOH; pH 6.0–7.0, 20 mM phosphate-NaOH; pH 7.0–8.0, 20 mM HEPES-KOH: pH 8.5–9.0, 20 mM Borax-H_2SO_4. Each buffer was supplemented with 100 mM mannitol. Partition of solutes into octanol or PGDP was determined with the shake-flask method [9].

Electrophoretic mobility of liposomes. Liposome suspensions (20 mg/mL, prepared as described previously [1]), 250 μL, were mixed with 375 μL of a solution containing 1.67 mM Proxicromil and 1 mM NaCl in phosphate-NaOH buffer at pH 6.0, for 2 hr with gentle shaking. This suspension, 125 μL, was then added to 24.9 mL phosphate-NaOH buffer at pH 6.0 and electrophoretic mobility was determined using a Zetasizer (Malvern Instruments, Malvern, U.K.). Mobility experiments were also performed at pH 4.5 and 6.0.

Statistical analysis. Observations were analysed to determine the mean and standard error of the mean

Table 2. Log P values for a series of non-ionizable solutes

Solute	Log P value		
	BBMV	Octanol	PGDP
Benzamide	0.21 ± 0.11 (8)	0.64	−0.36
Benzenesulphonamide	0.82 ± 0.04 (11)	0.31	−0.03
Methylphenylsulphone	0.88 ± 0.14 (9)	0.55	−0.41
Hydrochlorthiazide	0.91 ± 0.41 (8)	−0.1	−0.86
Methylphenylsulphoxide	0.98 ± 0.05 (14)	0.5	0.47
Aniline	1.04 ± 0.14 (14)	0.9	0.95
Phenylurea	1.04 ± 0.10 (11)	0.83	0.55
Phenyl benzamide	1.05 ± 0.10 (11)	2.62	2.62
Phenol	1.32 ± 0.11 (10)	1.46	1.42
Dimethylphenylsulphonamide	1.60 ± 0.08 (14)	1.35	1.28
Nitrobenzene	1.71 ± 0.16 (28)	1.85	2.11
Acetophenone	1.76 ± 0.07 (9)	1.58	1.60
Benzonitrile	1.81 ± 0.10 (13)	1.56	1.66
Benzylaldehyde	1.90 ± 0.05 (16)	1.48	1.57
Methylnaphthylsulphone	1.91 ± 0.04 (10)	1.74	2.00
Naphthylamide	1.99 ± 0.08 (12)	1.88	1.04
Naphthylsulphonamide	2.01 ± 0.13 (20)	1.55	1.28
Anisole	2.10 ± 0.12 (15)	2.10	2.22
Methylbenzoate	2.20 ± 0.08 (18)	2.11	2.1
Triphenylphosphineoxide	2.21 ± 0.08 (14)	2.27	1.56
3-(2-Napthoxy)-propylmethylsulphoxide	2.60 ± 0.09 (16)	2.42	2.56
Chrysene	2.60 ± 0.18 (12)	5.5	6.62
Fluoranthrene	2.61 ± 0.10 (7)	5.0	—
Toluene*	2.71 ± 0.25 (6)	2.69	2.8
Phenanthrene	2.75 ± 0.25 (13)	4.46	5.26
Naphthalene	2.78 ± 0.12 (12)	3.37	3.56
4-Chloro-1-naphthol	2.88 ± 0.11 (12)	3.3	3.22
1,2,5,6-Ibenzanthracene	3.09 ± 0.28 (15)	6.6	6.8
2-Methylanthracene	3.75 ± 0.22 (8)	5.0	5.22

BBMV values are means ± SEM for (N) determinations.

* Data from Ref. 1.

BBMV data were determined by HPLC determination of supernatant depletion as described in Materials and Methods. Solvent data (octanol and PGDP) were determined with the shake-flask method (see Materials and Methods).

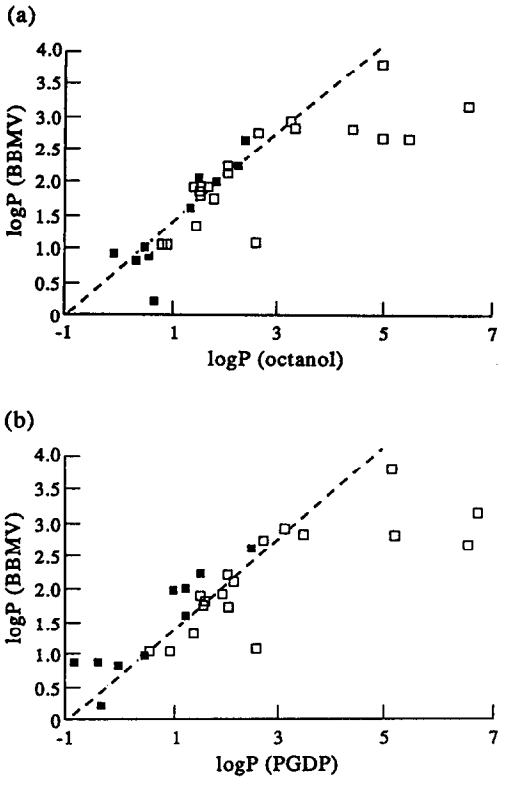

Fig. 1. Partition coefficients in BBMVs and model solvents for a range of solutes. Log P values for 29 solutes were determined in BBMVs, octanol and PGDP by the techniques described in Materials and Methods. BBMV log P value is plotted against (a) log P[OCT] and (b) log P[PGDP]. The lines were obtained by linear regression of data for log P[OCT] of log P[PGDP] <3.0. Strong proton acceptors are shown as solid squares.

(SEM). Significance of difference between mean observations was determined with a two-tailed Student's *t*-test using a statistical analysis system [10]. The level of significance was set at $P < 0.05$.

RESULTS

Partition of non-ionizable solutes into BBM

Previous work has demonstrated that the log P value determined in BBMVs represents partition into the lipid phase of the BBM and is independent of incubation time (>15 min) or temperature (10–40°), and the gender, age or small intestinal region of origin of the BBMVs [1]. A series of 29 non-ionizable solutes were investigated for partition into rat small intestinal BBM. The solutes were carefully chosen to include a range of chemical functionality likely to discriminate between solvent models of partitioning based on the linear solvation energy relationship model [11] for the relationship between structure and physical properties. Table 2 presents the results of these experiments, together with data for partition into octanol and the model solvent PGDP. Figure 1a and b shows the BBMV partition coefficients plotted against partition coefficients in octanol and PGDP, respectively. In both cases there is a linear relationship up to a log P of 3.0 although in both cases most of the polycyclic aromatic hydrocarbons (other than 3-methylanthracene) with log P > 3 distribute into BBMVs much less than expected. *N*-Phenyl benzamide is also much less lipophilic than expected (by about one log unit) in both model solvents. In addition, with the exception of benzamide, the BBMV partition coefficients of the most hydropholic solutes are underpredicted to a greater extent by PGDP than by octanol. Figures 1a and b also shows a hypothetical correlation line with a slope of approx. 0.7 and an intercept of −1. We have not reported a regression line for these data since, even with the exclusion of the polycyclic aromatics and *N*-phenyl benzamide as possible outliers, the slope and intercept of any regression would be very dependent on the leverage points of the benzamide and 3-methyl anthracene values. The hypothetical line is included to demonstrate an expected relationship based on a simple linear correlation between the effect of molecular structure on partitioning on the different solvent pairs. It has the added value of showing more clearly the key differences between the PGDP and octanol plots, which is the degree to which strong hydrogen bond acceptor solutes are found to be more hydrophilic as measured against PGDP than octanol. In comparing the octanol and PGDP plots, the underprediction seen for PGDP correlation arises from this effect. However, the converse is true for benzamide which is closer to the hypothetical line in PGDP. That the slope of the relationship, for both octanol and PGDP, is less than unity is normally taken to suggest that the BBMVs provide a "less hydrophobic" environment than either of the solvent models.

Partition of non-ionizable solutes into liposomes

Preliminary experiments with nitrobenzene demonstrated that an incubation time of 2 hr was required to reach equilibrium for partition into liposomes. The slower equilibration, compared to vesicles, may be attributed to the multilamellar structure of the liposomes. A group of eight solutes were investigated for partition into liposomes prepared from BBM lipids or purified lipids (Table 3). Figure 2 displays the linear relationship between log P determined in BBMVs and in BBM-derived liposomes. The linear regression line has a significant, non-zero intercept. Over log P values from 1.0 to >3.5, the line does not deviate significantly from the equality line log P [BBMV] = log P [BBM liposomes] and five of eight solutes show similar values in the two systems (including 2-methyl-anthracene, an outlier from the non-linear fit to BBMV and octanol partition data). Agreement between partition data in model liposomes and in BBM-derived liposomes was less good, with only two of five solutes studied showing similar log P values in the two systems (Table 3).

Partition of ionizable drugs in BBMVs and liposomes

The extent to which the ionized form of a drug extracts into a membrane is of great interest in drug design. Permeabilities are normally correlated with

Table 3. Partition coefficients for non-ionizable solutes in BBMVs, liposomes derived from BBMVs and model liposomes

Solute	Log P value		
		Liposomes	
	BBMVs	BBM lipids	Pure lipids*
Benzenesulphonamide	0.82 ± 0.04	0.03 ± 0.09	1.50 ± 0.14
Aniline	1.04 ± 0.14	0.98 ± 0.08	1.20 ± 0.10
Nitrobenzene	1.71 ± 0.16	1.45 ± 0.11	—
Naphthylamide	1.99 ± 0.08	1.99 ± 0.03	1.19 ± 0.09
4-Chloro-1-naphthol	2.88 ± 0.11	2.30 ± 0.12	—
Naphthalene	2.78 ± 0.12	3.50 ± 0.04	3.00 ± 0.22
2-Methylanthracene	3.75 ± 0.22	3.94 ± 0.13	3.98 ± 0.40
1,2,5,6-Dibenzanthracene	3.09 ± 0.28	3.08 ± 0.25	—

Values are means ± SEM for at least four determinations.
BBM lipids were extracted and liposomes prepared as described in Materials and Methods. Partition was determined as described in Materials and Methods.

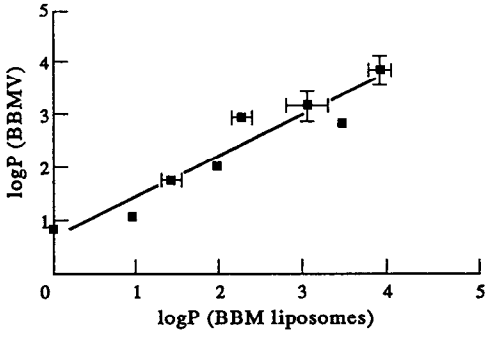

Fig. 2. Partition coefficients in BBMVs and liposomes prepared from BBM lipids. Log P values for eight solutes were determined in BBMVs and liposomes prepared from BBM lipids by the methods described in Materials and Methods. The line was obtained by linear regression.

Table 4. Partition of cationic drugs in octanol, BBMVs and BBM-derived liposomes at pH 7.4

Solute	Log D		
	Octanol	BBMVs	BBM liposomes
Atenolol	−2.5	1.36 ± 0.04 (14)	1.42 ± 0.1 (12)
Xamoterol	−1.5	1.46 ± 0.08 (8)	1.28 ± 0.07 (7)

Log D values [means ± SEM for (N) assays] were determined at pH 7.4 as described in Materials and Methods.

the fraction of unionized solute in the aqueous phase on the assumption that the extraction coefficient for the ionized form is insignificant by comparison. However, there have been attempts to enhance drug absorption by increasing the extraction of the ion-pair into the membrane and the question remains controversial [6].

In this work, we looked at the extraction of three model compounds, namely, the basic drugs Xamoterol® and Atenolol®, which are almost entirely in the cationic form at pH 7.4, and the acidic drug Proxicromil which is almost entirely anionic at that pH. To ensure that the results were not complicated by extensive protein binding, particularly since Proxicromil has been shown to bind to protein [12], partition studies for the two cations were carried out in both BBMVs and liposomes derived from BBM lipids (Table 4). Distribution into both systems for both the cationic drugs was found to be greater than that expected from their very low (< −1.5) octanol/water distribution coefficient. Partition studies with Proxicromil were carried out in BBM liposomes.

Figure 2 shows that a linear relationship exists between partition coefficients in the BBM vesicle and BBM lipid liposomes for eight non-protein binding solutes that were tested, demonstrating that BBM liposomes are a good model for the BBMV. Figure 3a shows the pH dependence of Proxicromil distribution into the BBM liposomes to be almost independent of pH in 0.15 M NaCl buffer. On reducing the NaCl concentration to 15 mM, a decrease in distribution coefficient with decreased unionized fraction (i.e. increasing pH) is observed (Fig. 3b). However, the slope of this line is much less (−0.43) than the theoretical line of −1.0 predicted by the neutral form partitioning alone. There is also the suggestion of a further decrease in slope at pH > 8.

To investigate the effect of salt concentration further we measured at pH 8 the effect on distribution of Proxicromil of increasing NaCl concentration between the two limits shown in Fig. 3a and b. Results (Fig. 4a) clearly showed the effect of NaCl concentration on distribution which was also seen in studies with LiCl (Fig. 4b). By contrast there is little effect of NaCl concentration on Proxicromil distribution when PGDP is the partitioning medium.

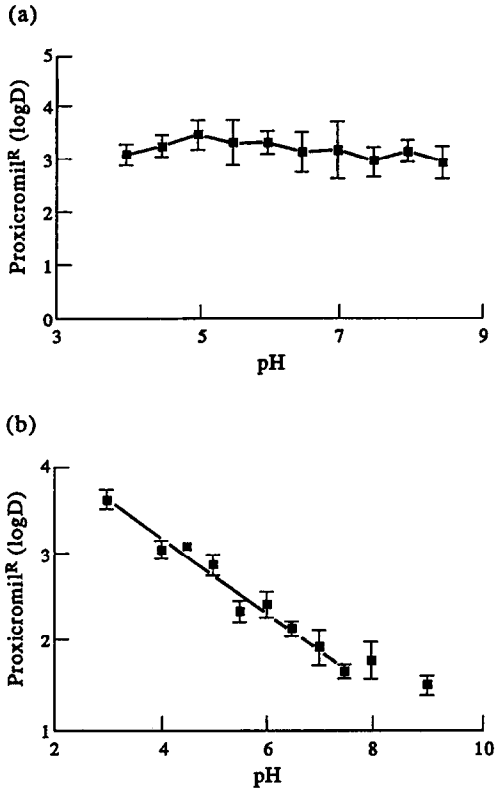

Fig. 3. Dependence of log D for Proxicromil in BBM liposomes on pH in (a) 0.15 M NaCl and (b) 0.015 M NaCl. Log P values for Proxicromil were determined in a series of buffers (see Materials and Methods for details) containing (a) 0.15 M NaCl or (b) 0.015 M NaCl. The line in (b) was obtained by linear regression of data for pH < 8.0.

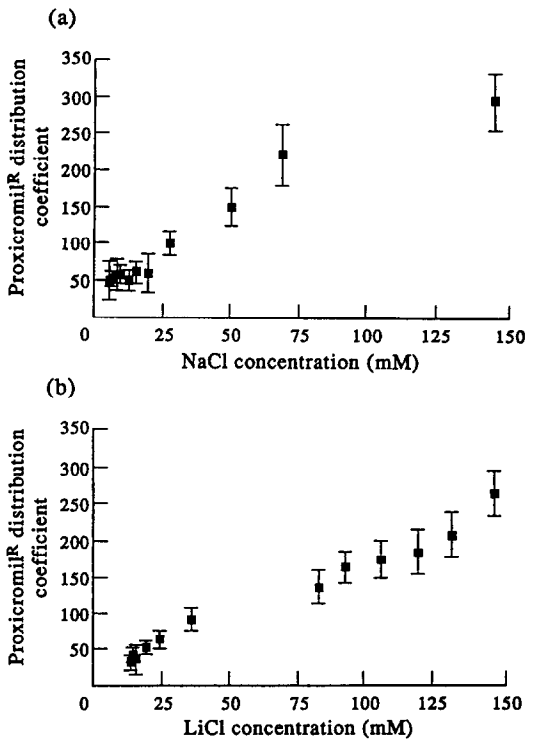

Fig. 4. Effect of increasing (a) NaCl and (b) LiCl concentration on partition of Proxicromil in PGDP at pH 8.0. Proxicromil distribution was determined in buffers with various NaCl concentrations at pH 8.0 as described in Materials and Methods.

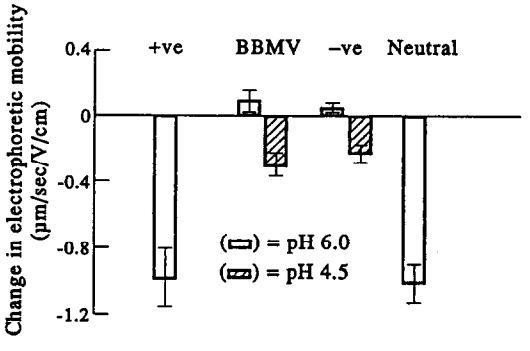

Fig. 5. Effect of Proxicromil on electrophoretic mobility of liposomes. Liposomes were prepared from purified lipid mixtures to yield various net surface charges (see Materials and Methods) or from BBM lipids. The change in electrophoretic mobility caused by addition of 1 mM Proxicromil was determined.

Effect of Proxicromil on electrophoretic mobility of liposomes

In order to better understand the nature of the species extracted into the membrane, a further set of experiments were carried out. The electrophoretic mobility of artificial liposomes of different compositions in the presence and absence of Proxicromil (1 mM) was determined. Results are shown in Fig. 5. The mobility of a liposome in an electrostatic field depends on both the size and the net surface charge. We measured the mobility of liposomes of BBM and three others which were neutral (egg lecithin/cholesterol, 1:1), positively (egg lecithin/cholesterol/stearylamine, 1:1:1) and negatively (egg lecithin/cholesterol/phosphatidic acid, 1:1:1) charged. Figure 5 shows the net change in mobility as a result of adding Proxicromil (1 mM). For the positively charged and neutral liposomes there is a very significant change in surface charge to become less positive or more negative, suggesting that the Proxicromil associated with the liposome membrane aligns such that its anionic group is at the surface. In contrast, at pH 6.0, BBM and the negatively charged liposomes seem to become less negative although the effect is barely significant. At pH 4.5 the lipid head groups of the BBM and negatively charged liposomes are much less ionized and therefore less negative, thus the initial effect of Proxicromil, as with neutral and positive liposomes, is to make the surface charge more negative.

DISCUSSION

Partitioning of non-ionizing solutes

A key difference between partitioning into organic solvents and lipid bilayers such as those found in

BBMVs is the highly ordered structure of the bilayer. The results suggest a hypothesis that the change in solute partitioning into BBMVs is roughly linear with that into the two organic solvents with a slope of about 2/3 and a non-zero intercept. The octanol correlation is more predictive, particularly for the strong proton acceptor solutes which are more hydrophilic in PGDP than in either BBMVs or octanol. The implication of this is that the solute is positioned within the bilayer so that it perceives a more mixed (octanol-like) hydrogen bond donor/acceptor environment. This is supported by a slope of less than unity which implies that the solutes experience an environment which is more polar than in either organic solvent. PGDP or a hydrocarbon solute would be expected to be a better model if the solutes were largely positioned in the hydrocarbon core of the bilayer. *N*-Phenyl benzamide cannot adopt the favourable position available to simple substituted benzenes where the polar group is aligned with the head groups of the lipids and the lipophilic group is also favourably positioned. There is no obvious explanation for the low value of benzamide, which does not fit this analysis.

The results seen for the polycyclic aromatics showing a maximum value in the BBMVs of between 2.5 and 3.0 were surprising. Previous work with dimyristoylphosphatidylcholine (DMPC) bilayers [13] also showed a plateau, although at a higher Log P (5.5) than in this work. DMPC bilayers would be expected to be much more fluid than BBMVs, which are a mixture of lipids and contain large amounts of cholesterol. In addition, DMPC liposomes are small and unilamellar, with high curvatures leading to increased disorder in the lipid chains. In both cases it seems likely that the polycyclic aromatic solutes are less lipophilic in BBMVs because of the disruption in the lipid bilayer core caused by their large volume. 3-Methyl anthracene does not fit this pattern with its BBMV value being much closer to that expected based on either octanol or PGDP. Presumably its linear structure allows a positioning in the bilayer which is much less disruptive than that of other, bulkier solutes.

Partitioning of ionizable solutes

The cationic drugs Xamoterol and Atenolol have significantly higher distribution coefficients in BBMVs (Table 2) than would be expected from either log P [OCT] or log P [PGDP]. Similar high values were observed in BBM liposomes suggesting that the lipid components of the membrane are responsible. The BBM lipids have a net negative surface charge and extraction of the cationic form into the membrane such that the cation aligns with the negatively charged head groups of the lipid is possible. Bilayer extraction has been proposed to explain Celiptum [14] and Tetracycline [15] partitioning to liposomes. Many workers have suggested that ionized solutes, in particular Proxicromil [16], penetrate membranes as the ion-pair and therefore intestinal drug absorption can be enhanced by manipulating the concentration and nature of the counter-ion [12, 16].

Figure 3a seems to support this view since partitioning of Proxicromil is independent of pH (as expected for an ion-pair) and much higher than would be expected based on the neutral form partitioning alone (log P [BBMV] approx. 0.5). We suggest that in this case both the neutral and the ionic forms distribute into the BBM liposomes, but the distribution of the anionic form is increased by increasing salt concentration. This may arise from ion-pair extraction, which is dependent on the Na^+ concentration, or it may be a simple salting out effect. The result with LiCl does not help clarify the situation since the sensitivity of distribution to LiCl concentration was no different.

The electrophoretic mobility experiments were designed to differentiate between ion-pair extraction and "surfactant" accumulation in membrane. The results in most systems suggest that there is a large increase in negative surface charge on adding Proxicromil which indicates that this solute extracts into the membrane as would a surfactant, rather than by ion-pairing. These results do not support the concept of ion-pairing enhancing absorption via increased extraction into membranes.

Partitioning and permeability

Our partitioning studies suggest that for both the monofunctional non-ionizable and the ionizable solutes the equilibrium position adopted in the membrane is that where the solute aligns with the lipid molecules such that ionic and polar groups are in the head region of the bilayer with the non-polar groups pointing towards the hydrocarbon core. It is the availability of this "surfactant-like" orientation that leads to the good correlation with octanol and the high partition values for both monocations and monoanions. This is important information and is consistent with partitioning studies by X-ray scattering [12] that show similar results. We caution that the observed correlation of BBMV partition coefficient with the amphiprotic, more polar octanol and the high extraction of ionized solutes may be misleading in terms of predicting permeability. Studies [17] of solute permeability tend to show better correlations with more hydrophobic solvents than octanol such as hydrocarbons and olive oil. Flux across a membrane requires partitioning into the hydrocarbon core and it is this equilibrium which is likely to dominate the permeability. Our results seem to be a measure of the equilibrium for the solute orientating into the bilayer and therefore represent only one step in permeation of a solute across a membrane.

Conclusions

(1) Octanol is a good model for drug partition into BBM for log P values up to 3.0. This is not necessarily the best model for permeability.

(2) Partitioning of ionizable drugs with BBM is much higher than expected from the organic solvent distribution due to "surfactant-like" ionic interactions with the membrane and its surface charges. Our results do not support the suggestion that ion-pairing enhances permeability.

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