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Facilitated transfer of cationic drugs across a lipoidal membrane by oleic acid and lauric acid

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Summary

The transfer of four β-adrenoceptor blocking agents of different lipophilicity across an artificial lipid membrane was measured using the rotating diffusion cells (RDC). A facilitated transport mechanism was established when oleic acid and lauric acid were incorporated in the membrane, using an appropriate pH gradient. The diffusion of metoprolol, oxprenolol and to a lesser extent propranolol was enhanced by an ion-pair mechanism in the presence of the fatty acids. The transfer of atenolol, the most hydrophilic of the drugs under investigation, was not enhanced. The results are useful in understanding the way in which charged molecules may be transported across biological membranes by ion pair mechanisms and also for the development of solid supported liquid membranes in separation technology.

Introduction

The permeation of charged species across lipophilic membranes purely by passive diffusion is very slow. This is a direct result of unfavourable partitioning into the membrane. However, transfer can be facilitated by the presence of carrier molecules in the membrane. Carrier-facilitated mechanisms have been identified in biological membranes and may be distinguished from active transport. It is therefore important to understand these in terms of basic physicochemical concepts to obtain a better understanding of membrane permeation and how charged drugs may be delivered across biological membranes.

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One mechanism by which charged species can be transported is by the formation of more lipophilic ion pairs. Examples have been documented in the literature (Barker and Hadgraft, 1981; Hadgraft et al., 1985). Many extraction processes utilising solid supported liquid membranes operate in a similar manner. In previous studies it has been shown that anionic species can be transported across an inert membrane impregnated with isopropyl myristate and that the carrier molecule is also capable of acting in vitro in excised skin (Hadgraft et al., 1986).

In a similar manner it is hoped to ion-pair four cationic β-blocking agents with the carboxylate anion of the fatty acids oleic acid and lauric acid (Table 1). The β-blockers were chosen since they cover a spectrum of lipophilic characteristics, and have similar structures and pK_a values around 9.5

(Table 1). Consequently, they will be predominantly cationic under physiological conditions. The lipophilicity of the β-blockers has been measured (Woods and Robinson, 1981) and found to fall into three categories: highly lipophilic, hydrophilic and intermediate in behaviour. Representative drugs from each group were chosen. From the first group propranolol hydrochloride and from the second atenolol were selected. Oxprenolol hydrochloride and metoprolol tartrate were chosen from the last.

The interfacial transfer kinetics of the various cationic drugs, from an aqueous donor phase across an IPM membrane, to an outer pH 7.4 phosphate buffer receptor, were measured using a rotating diffusion cell (RDC) (Albery et al., 1976). The effectiveness of the fatty acids as carriers was assessed by incorporating them in the IPM membrane. In order for the facilitated transport mechanism to operate, the pH of the donor phase should lie between the pK_a values of the cationic drug and the fatty acid. Under such conditions (Fig. 1) the carrier (A) will deprotonate at the donor compartment/membrane interface and ion-pair with the cation (HB^+) to form a neutral species ($HB^{+-}A$). The ion-pair will then diffuse, down its concentration gradient, to the opposing interface where it will release its cation.

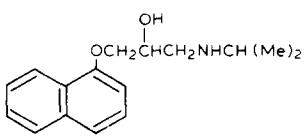
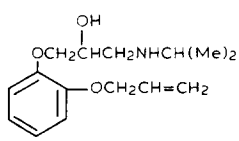
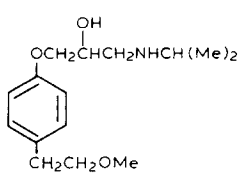
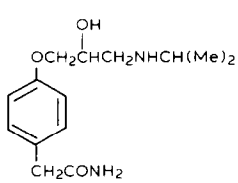
Materials and Methods

Materials

Propranolol hydrochloride and atenolol were gifts from ICI Pharmaceuticals plc (Macclesfield,

TABLE 1

Structural formulae, pK_a and spectroscopic data for the permeants and carriers

Chemical name	Structural formulae	pK_a (Moffat, 1986)	Wavelength of maximum absorption (nm)
Propranolol		9.5	289
Oxprenolol		9.5	274
Metoprolol		9.7	274
Atenolol		9.6	273
Oleic acid	$CH_3[CH_2]_4CH = CH[CH_2]_7CO_2H$	5.0 [a]	–
Lauric acid	$CH_3[CH_2]_{10}CO_2H$	5.0 [a]	–

[a] Approximate pK_a values (Kortüm et al., 1961).

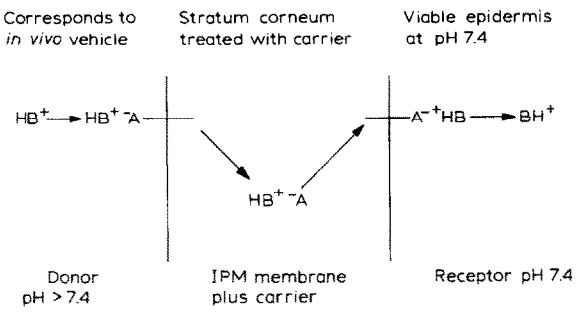


Fig. 1. Facilitated transport scheme for cationic drug molecules.

U.K.). Metoprolol tartrate and oxprenolol hydrochloride were gifts from Ciba-Geigy (Horsham, U.K.). All reagents were of GPR grade and supplied by BDH Chemicals, except for oleic acid and lauric acid which were biochemical grade. Isopropyl myristate was supplied by Croda Chemicals Ltd. Cellulose nitrate 0.2 μm pore size membrane filters were obtained from Whatman Ltd.

Determination of partition coefficients

Oil–water partition coefficients of the cationic drugs between equal volumes of IPM and aqueous buffer, at 32 °C, were obtained using a filter probe technique (Tomlinson, 1982) over a pH range 5.0–8.6.

The buffers were prepared using a combination of citric acid and phosphate buffer (McIlvane, 1980). The ionic strength of each buffer was adjusted to 0.6 M with sodium chloride. Both buffer and IPM were presaturated with each other. The concentration of the drug in the aqueous phase was monitored spectrophotometrically via a flow cell, at the wavelengths specified in Table 1. The partitioning experiments were performed on 3 different solutions in duplicate, making a total of 6 determinations of the partition coefficients for each drug. The effect of lauric acid and oleic acid on the partitioning of the drug was determined by the addition of 0.1 M of the fatty acid to the oily phase.

Measurement of interfacial rate constants

The flux of each cationic molecule across an IPM-impregnated membrane filter was measured down a concentration gradient using the RDC (Albery et al., 1976). The experimental techniques have been described in detail previously (Hadgraft et al., 1985). However, in this study a smaller pore size (0.2 μm) membrane filter was required in order to establish a stable oil–water interface in the presence of the carrier molecules. The donor and receptor compartments contained 40 cm^3 of phosphate buffer (pH 5.0–8.6) and 100 cm^3 of phosphate buffer (pH 7.4), respectively.

The flux of each drug was also measured against its concentration gradient. This was achieved by adding equimolar solutions of each drug to the donor and receptor phases in the RDC at the start of the experiment. To check that facilitated transfer was occurring against a concentration gradient a detailed study was not conducted and only one rotation speed, the fastest available, 4.67 Hz was used.

The results of the diffusion experiment were analysed either in terms of the flux of drug transported (J) or the apparent rate constant $\vec{k}$;

$$\vec{k} = J/A \cdot C_o$$

where A is the area of the filter and C_o the concentration of drug in the donor compartment.

Results and Discussion

The order of lipophilicity of the β-blockers, as evaluated by Woods and Robinson (1981) was confirmed by the partition coefficient as determined in an IPM/buffer system (Table 2). The partitioning of the cationic drugs into the IPM increased as the pH of the buffer increased. This is in accordance with the pH–partition theory since the amount of unionised drug increases with pH. Oleic acid increased the oil–water partition coefficient of propranolol hydrochloride over the whole pH range 5–8 (Fig. 2). The ratio of the partition coefficient into IPM plus oleic acid divided by that into IPM forms a maximum around pH 6.0. At this point ion-pairing is most effective as the drug exists predominantly in its cationic form and the fatty acids are also ionised. Lauric acid was as effective as oleic acid in increasing the pH-partitioning of propranolol. For an ionised

TABLE 2

Oil-water partition coefficients IPM/phosphate–citrate buffer at 32 °C: effects of oleic acid and lauric acid (± 95% confidence limits)

Drug	pH 7.4 between IPM/Buffer	pH 7.4 between 0.1 M oleic acid in IPM/Buffer	pH 7.4 between 0.1 M lauric acid in IPM/Buffer	pH 8.0 between IPM/Buffer
Propranolol hydrochloride	1.87 ± 0.09	51.63 ± 5.11	57.82 ± 6.29	7.33 ± 0.56
Oxprenolol hydrochloride	0.18 ± 0.02	5.67 ± 0.62	6.87 ± 0.71	0.45 ± 0.05
Metoprolol tartate	0.05 ± 0.01	1.87 ± 0.21	2.27 ± 0.23	0.14 ± 0.02
Atenolol	0.020 ± 0.004	0.030 ± 0.011	0.032 ± 0.004	0.024 ± 0.003

solute to pass from an aqueous to an organic phase it must initially overcome its hydration energy. The ability of the molecule to remain in the organic phase will be subsequently determined by ion-pair stabilization within the lipid environment. Atenolol is the most hydrophilic of the β-blockers, and hence subject to considerable hydration in the aqueous region, attempts to increase the partitioning through the formation of ion-pairs with both the fatty acids was low. Propranolol is highly lipophilic and will readily partition into IPM at high pH values, even in the absence of the fatty acids. At these pH values the ion-pair stabilization is not as significant in determining the partitioning behaviour of propranolol. Metoprolol and oxprenolol have more balanced partitioning behaviour, consequently at high pH values, the increase in partitioning brought about by the addition of fatty acids is expected to be more noticeable. At pH 7.4 the enhancement in partitioning in the presence of oleic and lauric acid was ca. 35 for oxprenolol and 41 for metoprolol (Table 2). Partitioning studies were not performed above pH 7.4 since the addition of fatty acids to the organic phase at pH 8.0 resulted in emulsification in the aqueous phase.

It was possible to transport propranolol, oxprenolol and metoprolol against their own concentration gradient within the rotating diffusion cell, provided that oleic acid or lauric acid were present in the membrane, and only when the pH of the donor phase exceeded that in the receptor phase. The flux values are given in Table 3. When the pH of the donor phase was less than 7.4 the

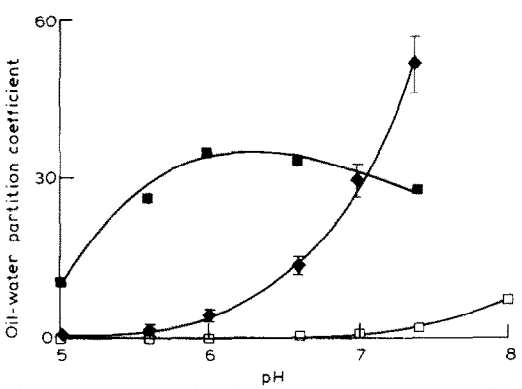


Fig. 2. The pH-partitioning behaviour of propranolol hydrochloride from phosphate–citrate buffer into IPM (□) and into 0.1 M oleic acid in IPM (◆). Each value is the mean ± 95% confidence limits. The ratio of partition coefficients between 0.1 M oleic acid in IPM divided by that into IPM is also shown (■).

TABLE 3

Flux of various cationic drugs against a concentration gradient

Drug	Donor phase pH	Flux ($mol \cdot mm^{-2} \cdot h^{-1}$) (± 95% confidence limits)	
		Membrane constituent	
		0.1 M oleic acid in IPM	0.1 M lauric acid in IPM
Propranolol	8.0	2.6×10^{-9} ($\pm 0.3 \times 10^{-9}$)	1.2×10^{-9} ($\pm 0.1 \times 10^{-9}$)
Oxprenolol	8.0	4.0×10^{-9} ($\pm 0.4 \times 10^{-9}$)	1.5×10^{-9} ($\pm 0.2 \times 10^{-9}$)
Metoprolol	8.6	3.9×10^{-9} ($\pm 0.5 \times 10^{-9}$)	1.2×10^{-9} ($\pm 0.2 \times 10^{-9}$)

The starting concentration of the donor and receptor phases (pH 7.4) were: propranolol hydrochloride 80 μM oxprenolol hydrochloride 400 μM, metoprolol tartrate 300 μM.

TABLE 4

Effect of fatty acids on the forward rate constant ($\vec{k}$) ($\pm$ 95% confidence limits) of various cationic drugs down a concentration gradient

Drug	pH (donor phase)	$\vec{k}$ ($\mu m \cdot s^{-1}$)		
		Membrane constituents		
		IPM	0.1 M oleic acid in IPM	0.1 M lauric acid in IPM
Propranolol hydrochloride	8.0	3.92 ± 0.45	6.94 ± 0.78	6.80 ± 0.79
Oxprenolol hydrochloride	8.0	0.22 ± 0.02	0.94 ± 0.11	0.75 ± 0.08
Metoprolol tartrate	8.6	0.13 ± 0.02	0.49 ± 0.05	0.39 ± 0.05
Atenolol	8.0–9.0	Transport not detectable		

β-blockers were transported in the opposite direction. In systems without fatty acids transfer occurred to a small extent ($\sim 2 \times 10^{-10}$ mol $\cdot$ $mm^{-2} \cdot h^{-1}$); however, this was at least an order of magnitude less than the facilitated flux.

In practice the drugs would transport down a concentration gradient and therefore the diffusion of the cations down their concentration gradients was studied at donor phase pH values greater than 7.4 but less than the pK_a of the β-blocker. The starting concentrations in the donor phase were 10 mM for propanolol hydrochloride and 25 mM for oxprenolol hydrochloride and metoprolol tartrate. The values for the forward rate constants of each cation (in order of lipophilicity) are presented in Table 4. The results show that the lipophilicity determines the flux magnitude i.e. propranolol is most readily transported. It is possible to enhance the transport of propranolol nearly two fold by the addition of oleic acid and lauric acid. Metoprolol and oxprenolol are enhanced approximately 4- and 3-fold by oleic acid and lauric acid, respectively. The flux of atenolol was not sufficient to be detected by the spectrophotometer in the pH range 8.0–9.0, despite the presence of the fatty acids.

In conclusion, the results obtained in this work suggest that various cationic drug transport can be facilitated across a lipid membrane, provided that a correct pH gradient is established and counter ions are present. This gradient could be utilised to enhance the absorption of cationic molecules across biological membranes and also in the concentration and separation of structurally related compounds using membrane technology.

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