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Stereoselective adsorption and trans-membrane transfer of propranolol enantiomers using cellulose derivatives

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Abstract

Cellulose tris(phenyl carbamate), cellulose tris(3,5-dimethylphenyl carbamate) and cellulose tris(3,5-dichlorophenyl-carbamate), which are known for their ability to resolve enantiomers when used as chromatographic stationary phases, were examined in terms of their ability to adsorb differentially the enantiomers of propranolol hydrochloride and consequent differential transfer when used as excipients in aqueous donor vehicles. Over a range of conditions investigated, optimum stereospecificity was found at pH 7.4, an incubation temperature of 32°C and propranolol concentration of 1 $mgml^{-1}$ with 1 mg adsorbent. The ratios of S/R bound was 1.35, 2.65 and 2.31, respectively. The permeation rates of propranolol enantiomers were determined through Silastic membrane in the presence and absence of vehicular cellulose tris(3,5-dimethylphenyl carbamate) and cellulose tris(3,5-dichlorophenyl carbamate). When pure propranolol enantiomers were used, permeation rates of propranolol enantiomers were significantly different, both numerically and statistically (steady-state flux ratios, R/S = 1.70 and 1.68; $P = 0.03$ and 0.001, respectively). In the absence of adsorbent permeation rates were not significantly different. When racemic propranolol was used, the flux ratios were less, but still of statistical and numerical significance (steady-state flux ratios, R/S 1.14 and 1.14; $P = 0.04$ and 0.01, respectively) considering the differential activities of propranolol enantiomers. These results demonstrate the potential of enantioselective retardation in transmembrane transfer as an alternative methodology for administering single enantiomers.

Keywords: Stereoselective adsorption; Stereoselective retardation; Propranolol; Enantiomers; Cellulose derivatives; Transmembrane transfer

1. Introduction

Propranolol is the most commonly prescribed β-blocker and is currently administered as a racemate, a 50:50 mixture of two enantiomers, with

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the eutomer, S-(l)-propranolol having 100-fold greater activity than the distomer, R-(d) propranolol (Barett and Cullum, 1968). As the distomeric activity is relatively benign the administration of the racemic form of this drug is currently accepted, although, as with many other drugs, this may not remain the case indefinitely in view of the increasing pressure from regulatory bodies for the provision of single stereoisomers. However, administering stereochemically pure propranolol, or indeed any other chiral drug, would involve bulk scale processes, such as chromatographic separation or asymmetric synthesis, both of which have shortcomings with regard to cost and difficulty. A potential alternative approach for administering single enantiomers involves the formulation of a racemate with a compound capable of stereoselective adsorption, such that one enantiomer is preferentially bound whereas the other is released.

To this end, three compounds based on derivatised cellulose (Fig. 1) were synthesised and examined in terms of the relative adsorption characteristics and membrane permeation of the enantiomers of propranolol hydrochloride under static, aqueous conditions. The enantioselectivity of derivatives of cellulose is well recognised and early reports described useful resolutions by stationary phases containing cellulose triacetate (Hesse and Hagel, 1976) and cellulose trisphenylcarbamate (Okamoto et al., 1984). Further improvements were obtained when the phenyl groups were substituted, particularly in the 3 and 5 positions of the phenyl ring (Okamoto et al., 1986a). In particular, the compounds cellulose tris(3,5-dichlorophenyl carbamate) and tris(3,5-dimethylphenyl carbamate) were found to exhibit the greatest resolving capability for a wide range of racemic compounds, including β-blockers such as propranolol, the latter being commercially available as a HPLC stationary phase in either normal or reverse phase. The aim of this work was to test the hypothesis that the chromatographic resolution of racemic propranolol by these compounds would be reflected in differential adsorption properties and rates of permeation across a model membrane. Polydimethylsiloxane (Silastic) membranes have been used extensively since they being proposed as model biological membranes (Garrett and Chemburkar, 1972).

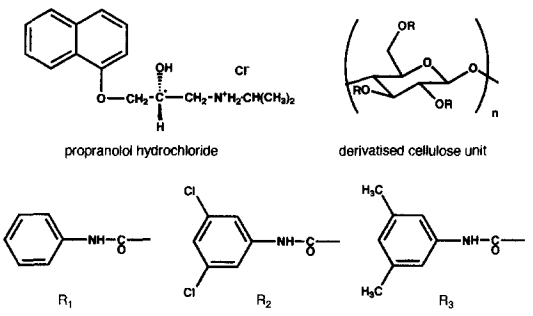


Fig. 1. Structures of propranolol hydrochloride and derivatised cellulose, where R_1 = phenyl carbamate, R_2 = 3,5-dichlorophenyl carbamate and R_3 = 3,5-dimethylphenyl carbamate.

The permeation rates of propranolol enantiomers have been studied previously across rat skin (Miyazaki et al., 1992; Heard et al., 1993a), human skin (Heard et al., 1993b) and Testskin (Touitou et al., 1993) with regard to intrinsic stereoselectivity. Stereoselective vehicular complexation was proposed as a mechanism for the observed differential permeation of hyoscyamine enantiomers across human skin (Whomsley et al., 1993). Modest stereoselective release has been reported from formulations containing racemic propranolol and the film forming excipient hydroxy

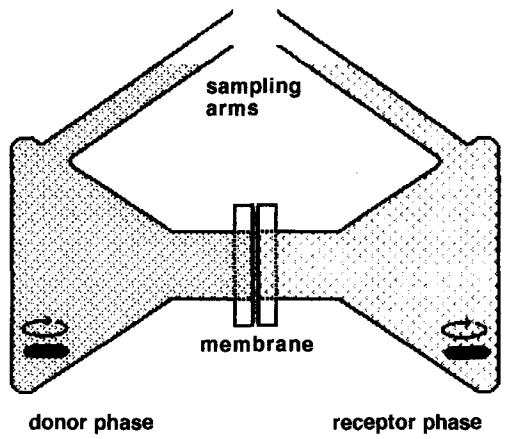


Fig. 2. Diffusion cell set up used to measure membrane transport. The membrane is presented vertically to allow stirring of both donor and receptor phases, thereby preventing settling of solid adsorbents and minimising stagnant layer effects. Sampling arms were offset to enable addition of solutions and sampling.

methylpropyl cellulose (Duddu et al., 1993). Greater stereoselective release of the enantiomers of tiaprofenic acid, both in vitro and in vivo, was recently reported from drug/derivatised β-cyclodextrin mixtures (Vakily et al., 1995). Significant stereoselective adsorption of a drug enantiomer would effectively produce a decreased vehicular concentration of the bound enantiomer, which should manifest itself as decreased permeation rates, hence lower bioavailability. We believe this to be the first report to view such phenomena as a potential means of deliberate stereospecific retardation of one enantiomer in the formulation of drug racemates.

2. Materials and methods

2.1. Materials

R-(d)- and S-(l)-propranolol HCl were obtained from Cambridge Research Biochemicals, Northwich, Cheshire, UK. The enantiomeric purity of S-(l)-propranolol HCl was found to be 95% and that of R-(d)-propranolol HCl 93%, using the method described below. ± propranolol hydrochloride was a gift from ICI Chemicals, Macclesfield, Cheshire, UK. Microcrystalline cellulose was obtained from Merck, Poole, Dorset, UK. Phenyl isocyanate, 3,5-dimethyl isocyanate and 3,5-dichloro isocyanate were purchased from Lancaster Synthesis. All other chemicals were AnalaR grade or equivalent. Silastic sheet, thickness 0.3 mm and hardness 60°SHR was obtained from Samco Silicone Products, St Albans, Hertfordshire, UK.

2.2. Synthesis of cellulose tris(phenyl carbamate), cellulose tris(3,5-dimethylphenyl carbamate) and cellulose tris(3,5-dichlorophenyl carbamate)

Cellulose tris(phenyl carbamate) derivatives were prepared using a previously described method (Okamoto et al., 1984), whereby microcrystalline cellulose (0.005 M) was reacted with an excess (0.017 M) of phenylisocyanate, 3,5-dimethyl isocyanate and 3,5-dichloro isocyanate in pyridine at 120°C for 6 h. Reaction mixtures were then poured into methanol to remove unreacted isocyanate. The crude product was washed several times with methanol, filtered and dried. Yields were > 95%. The compounds were characterised by IR, NMR, MS and polarimetry which confirmed virtually stoichiometric derivatisation of each glucose unit.

2.3. Adsorption studies

The binding of the enantiomers of propranolol to cellulose tris(3,5-dimethylphenyl carbamate) and cellulose tris(3,5-dichlorophenyl carbamate) in aqueous conditions was determined at five drug concentrations and at two temperatures: ambient and 32°C. Two pH values were employed, 5.5 and 7.4, to represent physiological pH and the pH gradient that exists across the stratum corneum. Solutions of each enantiomer were prepared in a phosphate/citrate buffer at concentrations of: 0.1, 0.2, 0.5, 1.0 and 1.20 mg ml^{-1}. Ten mg of cellulose compound was weighed directly into vials followed by 1 ml aliquots of the relevant solution. Samples were incubated for 24 h and the unbound drug separated from the adsorbed drug/adsorbent by ultrafiltration (Amicon MPS-1, Danvers, MA, USA). Pure enantiomers were used in order to monitor for racemisation during the duration of the experiment. Each experiment was performed in triplicate. The stability of R-(d)- and S-(l)-propranolol HCl was also determined under the conditions used as well as the time taken to attain equilibrium binding.

2.4. Membrane permeation studies

Silastic sheet was washed, cut into 2-cm squares and mounted in a modified Franz-type diffusion cell, consisting of two horizontal receptor compartments with the membrane presented vertically (Fig. 2). This arrangement was necessary to allow continuous donor phase agitation, thereby keeping the adsorbent in suspension and, at the same time, minimising stagnant layer effects. One hundred μl of 1 $mgml^{-1}$ racemic, R-(d)- or S-(l)-propranolol HCl was the added 0.1 g of tris(3,5-dimethylphenyl carbamate) or tris(3,5-dichlorophenyl carbamate) in 2.0 ml of pH 7.4

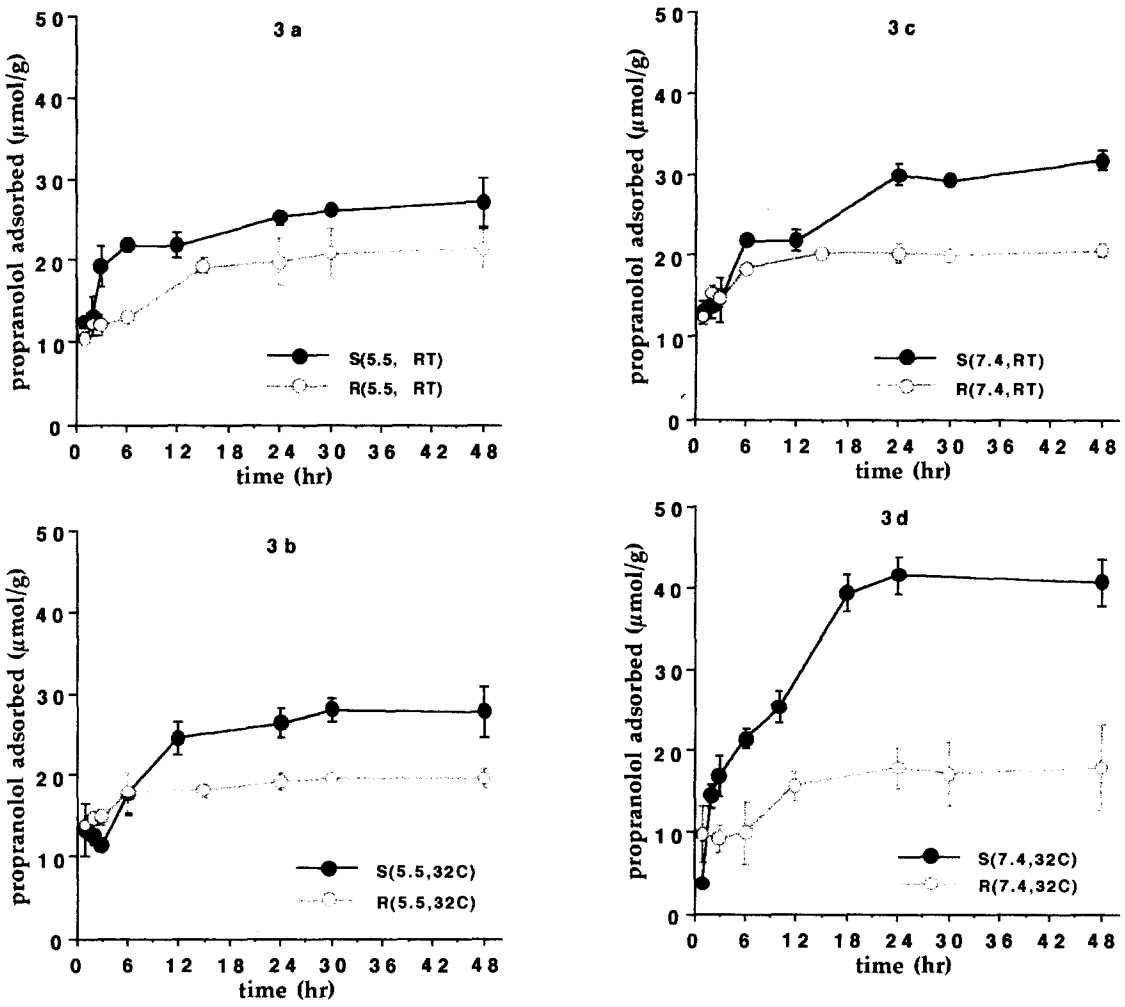
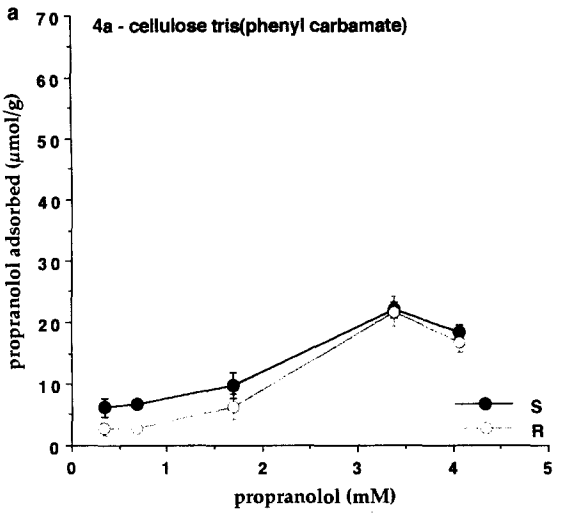


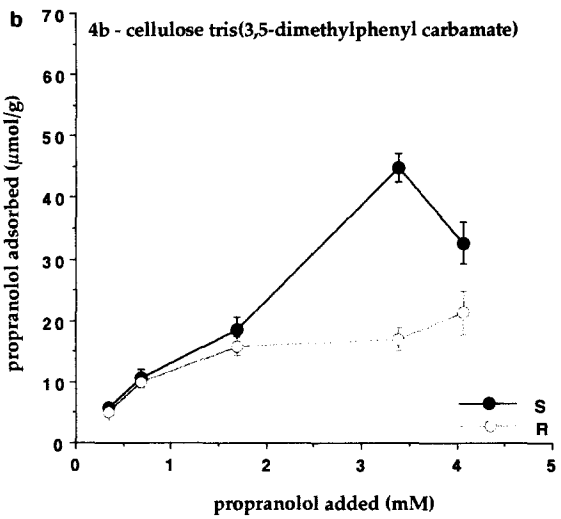
Fig. 3. Time plot of R and S-propranolol HCl adsorbed on cellulose tris(3,5-dimethylphenyl carbamate), under various conditions: (a) pH 5.5, room temperature; (b) pH 5.5, 32°C; (c) pH 7.4, room temperature; (d) pH 7.4, 32°C.

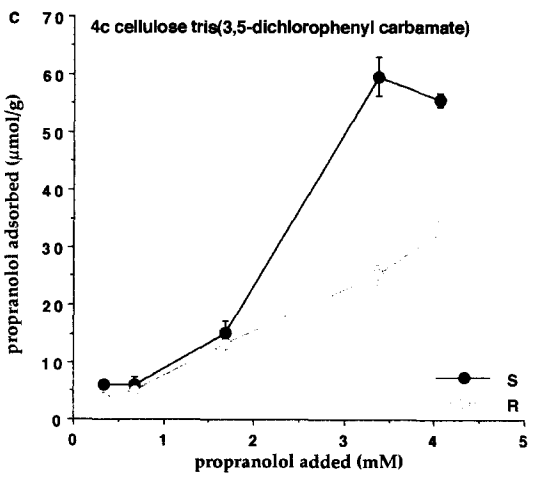
buffer and pre-equilibrated and stirred for 24 h at 32°C. This mixture was added to the donor phase of the diffusion cell simultaneously with 2 ml of pH 7.4 buffer which was added to the receptor phase. The cells were then placed in a water bath set at 32°C, 200-μl samples taken at appropriate timepoints between 0–96 h and analysed by chiral HPLC. Each experiment was performed in triplicate. Control experiments were carried out in which no adsorbent was present in the donor phase.

2.5. Analysis

Samples were analysed directly by reverse-phase chiral HPLC. The system consisted of a Marathon II autosampler, Milton Roy CM4000 pump, Milton Roy UV detector SM4000 (measuring at 289 nm) and chromatograms were recorded on a LDC Cl-4100 integrator. A flow rate of 0.5 ml min^{-1} was used throughout. Two chiral columns were used: Daicel OD-R (mobile phase 60:40 1 N sodium perchlorate: acetonitrile; typical







retention times were 9.1 min for R-(d)-propranolol and 11.9 min for S-(l)-propranolol) and Chromtech AGP (mobile phase 0.5% propanol in 110 mM pH 4.1 ammonium acetate buffer; typical retention times were 9.6 min for R-(d)-propranolol and 13.4 min for S-(l)-propranolol. The former was used to assay samples containing pure enantiomers and the latter was used for samples containing ± propranolol, where increased sharpness of the S-enantiomer peak was required.

3. Results

3.1. Adsorption

Fig. 3a–d are plots of the quantities of propranolol enantiomers adsorbed by cellulose tris(3,5-dimethylphenyl carbamate) over a period of 48 h over a range of conditions. Under all conditions employed, stereoselective binding occurred within 6 h of incubation and equilibrium was attained within 24 h. In all cases, it was the S-enantiomer that was preferentially bound. Incubation at 32°C appeared to increase differential binding and the greatest differential binding occurred at pH 7.4 and incubation at 32°C. All subsequent experiments were performed under these latter conditions. As the other adsorbents were of similar chemical structure, it was assumed that their equilibrium binding characteristics were similar. The optical purity of both enantiomers remained constant throughout all experimental work. Fig. 4a, b and c show the effect of propranolol concentration on binding. In Fig. 4a, the adsorbent was cellulose tris(phenyl carbamate) and the amount adsorbed was maximal at an initial concentration of 1.0 $mgml^{-1}$. Although statistically insignificant, there was a tendency for more S-propranolol to adsorb than R propranolol. In the presence of tris(dichorophenyl carbamate) (Fig. 4b) and tris(dimethylphenyl carbamate) (Fig. 4c), binding was much increased and at higher concentration

Fig. 4. Steady state flux of the single enantiomers of propranolol HCl across Silastic membrane in presence and absence of cellulose triphenyl carbamate derivatives.

Table 1
Differential adsorption of S-(l)- and R-(d)-propranolol hydrochloride (3.38 mM) by cellulose derivatives in pH 7.4 buffer at 32°C (n = 3, ± standard error)

Adsorbent	R-enantiomer (μmol g^{-1})	R-enantiomer (μmol g^{-1})	Ratio S/R
Cellulose tris(phenyl carbamate)	21.86 ± 0.52	16.20 ± 1.23	1.35
Cellulose tris(3,5-dichlorophenyl carbamate)	59.6 ± 3.41	25.83 ± 1.72	2.31
Cellulose tris(3,5-dimethylphenyl carbamate)	44.73 ± 2.30	16.90 ± 1.85	2.65

Table 2
Steady state flux, flux ratio and lag time for permeation of optically pure R and S propranolol across Silastic at pH 7.4 and 32°C (n = 4, ± standard error)

Adsorbent		Flux (μgcm^{-2} h^{-1})	P	Ratio R/S	Lag time (h)	P
Control	R	0.82 ± 0.11	0.98	0.99	22.18 ± 3.00	0.17
	S	0.83 ± 0.21			25.45 ± 0.50	
Cellulose tris(3,5-dimethylphenyl carbamate)	R	0.75 ± 0.12	0.03	1.70	32.49 ± 9.06	0.21
	S	0.44 ± 0.11			24.47 ± 2.50	
Cellulose tris(3,5-dichlorophenyl carbamate)	R	0.84 ± 0.03	0.001	1.68	40.77 ± 2.30	0.58
	S	0.50 ± 0.03			41.62 ± 0.75	

levels there was a clear stereospecific trend in that significantly more of the S-enantiomer was binding than the R-enantiomer. This again appeared to be maximal at a concentration of 1.0 mgml^{-1}, beyond which the difference between the two

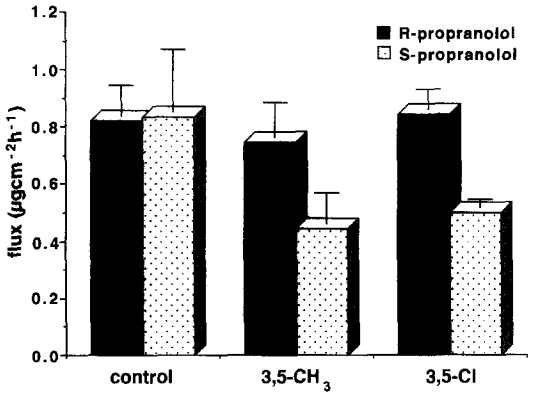


Fig. 5. Histogram of steady-state flux of optically pure R- and S-propranolol hydrochloride across Silastic (pH 7.4, 32°C).

enantiomers appeared to diminish. Table 1 summarises the differential adsorption observed at a drug concentration of 1.0 mgml^{-1}. It can be seen that cellulose tris(phenyl carbamate) demonstrated significant stereospecificity. Cellulose tris(3,5-dichlorophenyl carbamate) was more stereospecific (ratio S/R 2.31), but bound a greater proportion of both enantiomers. Cellulose tris(3,5-dimethylphenyl carbamate) demonstrated the greatest stereospecific binding (ratio S/R 2.65).

3.2. Membrane diffusion — single enantiomers

Table 2 gives steady-state flux values when the individual enantiomers of propranolol were added to the donor phase. These data are also presented as histograms (Fig. 5). In control experiments (no stereospecific adsorbant), the diffusion of the two enantiomers of propranolol demonstrated no statistically significant differences ($P < 0.05$). In the presence of cellulose tris(3,5-dichlorophenyl-

Table 3
Steady state flux, flux ratio and lag time for transfer of R and S propranolol from racemate across Silastic at pH 7.4 and 32°C (± standard error)

Adsorbent		Flux ($\mu gcm^{-2}\ h^{-1}$)	*P*	Ratio R/S	Lag time (h)	*P*
Control[a]	R	0.44 ± 0.06			10.69 ± 1.82	
			0.88	0.98		0.41
	S	0.45 ± 0.06			12.07 ± 1.88	
Cellulose tris(3,5-dimethylphenyl carbamate)[b]	R	0.40 ± 0.05			16.39 ± 0.94	
			0.04	1.14		0.76
	S	0.35 ± 0.04			16.85 ± 2.26	
Cellulose tris(3,5-dichlorophenyl carbamate)[c]	R	0.42 ± 0.02			16.73 ± 2.09	
			0.01	1.14		0.24
	S	0.37 ± 0.03			13.44 ± 3.52	

[a] n = 4.
[b] n = 7.
[c] n = 6.

carbamate), the flux rate of R-propranolol was almost twice that of S-propranolol (flux ratio 1.7, $P = 0.001$) indicating that the latter was more strongly adsorbed than the former. Similarly, in the presence of cellulose tris(3,5-dimethylphenyl carbamate) there were significant differences in flux rates (flux ratio 1.68, $P = 0.03$), although increased binding of S-propranolol was also apparent. No significant differences were found in lag times.

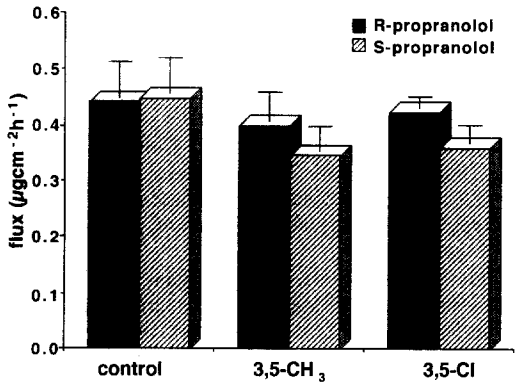


Fig. 6. Histogram of steady-state flux of R- and S-propranolol hydrochloride across Silastic (pH 7.4, 32°C) from racemate.

3.3. Membrane diffusion — racemate

Table 3 and Fig. 6 show the flux data of the enantiomers of propranolol when the racemic drug was added to the donor phase. In control experiments (no stereospecific adsorbant), the diffusion of the two enantiomers of propranolol again demonstrated no statistically significant differences (note, flux values approximately half those of Table 1 due to halving the concentration of each enantiomer). In the presence of cellulose compounds, the diffusion rate of S-propranolol was lower than R-propranolol, although the differences were much reduced relative to the results obtained for pure enantiomers. For tris(3,5-dichlorophenyl carbamate), the flux ratio was 1.14 ($P = 0.01$); for tris(3,5-dimethylphenyl carbamate) the flux ratio was also 1.14 ($P = 0.01$). No significant differences were found in lag times.

4. Discussion

Stereospecific interaction between cellulose tris(3,5-dimethylphenyl carbamate) and cellulose tris(3,5-dichlorophenyl carbamate) with propranolol enantiomers under static aqueous conditions was confirmed in terms of both differential ad-

sorption and differential transfer across Silastic membrane. The ratio of S/R bound was consistent with the elution order typically experienced with such materials when used as chromatographic stationary phases (Okamoto et al., 1986a), such that S-propranolol clearly has greater affinity for these compounds than R-propranolol. The use of optically pure propranolol enantiomers followed by chiral HPLC was a convenient in-built check for racemisation; no racemisation was found over the duration of the experiments.

Adsorption was found to reach equilibrium within 24 h. Adsorption is a kinetic process and sufficient time must be allowed for Brownian motion and binding interactions to attain equilibrium. The observation that differential binding diminished after a maximum was unexpected, but may indicate that beyond the optimum solute concentration the presence of excess drug impeded binding site docking. Over the pH range examined (7.4 and 5.5), the degree of ionisation of the drug (pk_a = 9.2) would not significantly change. In addition, increasing the incubation temperature from ambient to 32°C would increase the Brownian motion of the solution but also increase the dissociation of drug from binding site. Hence, the net effect of these parameters did not appear to be significant.

In the absence of cellulose excipient, the diffusion of propranolol through Silastic was found to be not stereoselective, as anticipated. The much reduced diffusion rate of S-propranolol relative to S-propranolol must therefore have been due to the differential affinities for the cellulose adsorbents and, in compliance with Fick's first law of diffusion, the different apparent donor phase concentrations. However, these considerable differences were not as marked when ± propranolol was used, although the trend was the same and results were of statistical significance. The reasons for this are unclear, but again may have been due to competition in the vicinity of the adsorbent binding sites, or possibly a complexation effect. There have been several investigations into the stereoselective nature of these cellulose compounds (Okamoto et al., 1986a; Okamoto et al., 1986b), although the precise mechanism remains to be elucidated.

To summarise, the use of enantioselective retardation as a means of administering single enantiomers from a racemate has been demonstrated in principle. Although the level of stereoselectivity demonstrated in this case was generally quite small, the results were of significance, particularly when considering the differential activities of the two enantiomers of propranolol. Clearly, the adsorbent compounds investigated here are incapable of complete retention of the distomer, indeed, in the case of propranolol it was the eutomer that was prefentially bound. However, if the features responsible for the proven stereospecificity of these adsorbent compounds can be identified and enhanced, a workable system, in which a distomer is maximally bound, should be an achievable goal.

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