J. Pharm. Pharmacol. 1998, 50: 63–69
Received August 1, 1997
Accepted September 11, 1997

The Effect of Protein Binding on the Hepatic First Pass of *O*-Acyl Salicylate Derivatives in the Rat

DANIEL Y. HUNG, GEORGE D. MELLICK, BENJAMIN D. WHITEHEAD AND MICHAEL S. ROBERTS

Department of Medicine, The University of Queensland, Princess Alexandra Hospital, Woolloongabba, Queensland, Australia

Abstract

In this work the in-situ perfused rat liver has been used to examine the effect of changing the protein content of the perfusate on the hepatic extraction of *O*-acyl esters of salicylic acid. The hepatic availability (F) of these solutes was studied at a flow-rate of 30 mL min^{-1} with perfusate albumin concentrations of 0, 2, and 4% w/v.

The hepatic availability of the esters was shown to decrease with increasing carbon-chain length in the *O*-acyl group; for all the esters the hepatic availability increased with increasing albumin concentration in the perfusate. The dispersion-model-derived efficiency number (R_N) of the esters was shown to increase with increasing lipophilicity and decrease with increasing albumin concentration in the perfusate. The unbound fraction (f_u) of the esters decreased with lipophilicity. R_N/f_u for acetylsalicylic acid remained relatively constant as the albumin concentration was increased. However, R_N/f_u for *n*-pentanoyl- and *n*-hexanoylsalicylic acids increased significantly as albumin concentration increased from 0% to 4%.

Thus, for the more lipophilic solutes (*n*-pentanoyl- and *n*-hexanoylsalicylic acids) the presence of albumin apparently facilitates the uptake of unbound solute relative to acetylsalicylic acid.

It is generally accepted that only the unbound fraction of a solute is available for uptake into hepatocytes. However, for several highly protein-bound organic anions such as taurocholate (Forker & Luxon 1981), rose bengal (Forker & Luxon 1983), dibromosulphophthalein (Sluijs et al 1987), fatty acids (Schwab & Goresky 1991) and oleate (Weisiger et al 1987) the increase in the hepatic uptake of a solute with increasing protein concentration exceeds expectations based on the unbound fraction. This observation implies that the binding protein in some way 'facilitates' solute uptake into the hepatocytes.

Several approaches have been proposed to explain the 'pseudo' facilitation of solute uptake by albumin. An albumin receptor model was postulated by Weisiger & Ma (1981) to explain the dependence of the kinetics of oleate uptake by the perfused liver on the concentration of the albumin-oleate complex rather than on the concentration of free oleate. The observations of saturable binding of labelled albumin to isolated hepatocytes supported the suggestion of an albumin receptor. Attempts to isolate and characterize an albumin receptor, however, have been unsuccessful (Stremmel et al 1983). An alternative protein-dissociation-limited diffusion model for kinetics of albumin uptake by hepatocytes, that does not require the presence of an albumin receptor, has been discussed extensively (Weisiger et al 1984; Weisiger 1985; Sluijs et al 1987; Weisiger & Ma 1987). This approach assumes that the uptake of free ligand by the cell is very fast such that the dissociation of ligand from the complex never reaches equilibrium and the amount of bound ligand becomes rate-limiting. However, this model explains neither the saturable binding of albumin to hepatocytes nor the cell-mediated acceleration of dissociation of albumin-palmitate complexes (Reed & Burrington 1989). The possible contribution of diffusion through the unstirred water layer to the albumin-mediated transport phenomenon was initially proposed by Bass & Pond (1987). This theory assumes the existence of an unstirred water layer adjacent to the surface of the hepatocyte. If the effect of the unstirred water layer was considered, previous data that indicate albumin-medi-

Correspondence: M. S. Roberts, Department of Medicine, The University of Queensland, Princess Alexandra Hospital, Woolloongabba, Queensland, Australia.

ated transport could be explain mathematically. However, direct anatomical measurements of the size of the unstirred water layer in the intact sinusoid have yet to be determined. Morgan et al (1990) suggested that the kinetic analysis used by others was inappropriate. They used a kinetic model which incorporated the unbound fraction of ligand into the general model for diffusion between two compartments and showed that the apparent rate-constant for unbound ligand concentration increased with albumin concentration, although the uptake clearance of unbound ligand remained constant.

The major aim of this study was to determine whether changes in hepatic elimination with changing protein content are explained by a conventional approach or whether an albumin-mediated transport process ('pseudo' facilitation) is implied.

We have recently synthesized and characterized a series of *O*-acyl derivatives of salicylic acid for which protein binding increases with increasing carbon number in the ester side-chain (Hung et al 1997). The hepatic disposition of these solutes in the perfused rat liver has also been reported for protein-free perfusate (Mellick et al 1997). In this paper we describe the influence of changing perfusate protein content on hepatic elimination of these *O*-acyl salicylate derivatives: acetylsalicylic acid (aspirin), *n*-pentanoylsalicylic acid and *n*-hexanoylsalicylic acid (Figure 1). The hepatic availability (F) of these esters was determined in the single-pass in-situ perfused rat liver under conditions of 0, 2, and 4% bovine serum albumin (BSA) in the perfusate. The efficiency number (R_N) which characterizes the elimination of these esters by the liver was then derived from the dispersion model analysis. Subsequently, R_N/f_u was plotted against albumin concentration to determine whether the removal of these solutes by the liver, when corrected for the unbound fraction of solute, remained constant or increased as albumin concentration was increased.

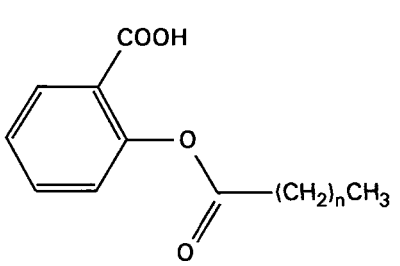

Figure 1. Structures of *O*-acyl derivatives of salicylic acid. The derivatives differ only in the length of the side chain (i.e. the value of n).

Materials and Methods

Synthesis

The esters were synthesized by a procedure outlined elsewhere (Hung et al 1997). Briefly, the *O*-acyl derivatives of salicylic acid were prepared by esterification of the acid with the appropriate acyl anhydride. The products were recrystallized from mixtures of acetone and petroleum spirit or water and ethanol. Their structures were confirmed by infrared, nuclear magnetic resonance and mass spectroscopy. Differential scanning calorimetry was used to assess the purity of each compound (> 98% for all esters).

Analytical

High-performance liquid chromatography (HPLC) was performed with a double piston pump (Shimadzu, model LC-6AD), an autoinjector (Shimadzu, model SIL-6B), a variable-wavelength UV-detector (Waters-Millipore, model Lambda-Max 481), a system controller (Shimadzu, model SCL-6B), a Delta personal computer and a 250 mm × 4·6 mm i.d. × 5 μm Brownlee C_{18} reversed-phase column (Applied Biosystems).

The HPLC method used for the determination of the *O*-acyl salicylic acid derivatives has been outlined elsewhere (Hung et al 1997). The mobile phase used for acetylsalicylic acid was 29 : 70 : 1 acetonitrile–0·03% (v/v) phosphoric acid–triethylamine at pH 2·0; the flow-rate was 1 mL min^{-1}. *p*-Toluic acid (0·004% w/v) was used as internal standard. For *n*-pentanoyl- and *n*-hexanoylsalicylic acids the mobile phase was 49 : 50 : 1 acetonitrile–0·03% (v/v) phosphoric acid–triethylamine at pH 2·0; the flow-rate was 1 mL min^{-1}. For these solutes diflunisal in acetonitrile (0·004% w/v) was used as internal standard. The effluent was monitored at 237 nm and the sensitivity range was 0·04 aufs. In these solvent systems salicylic acid could be also assayed, with good separation from the other solute peaks.

The within day coefficients of variation for all the compounds were within the range of 2·52–4·76% (n = 3).

Protein binding

These experiments were performed in Krebs–Henseleit buffer (pH 7·4) containing 2% or 4% w/v bovine serum albumin (BSA) and incubated at 37°C in a water bath for 30 min to reach the required temperature. A 500 μM solution of each salicylate ester was prepared at each protein concentration.

The unbound fraction (f_u) of parent drug and its hydrolysis product (salicylic acid) were investigated using an ultra-filtration method. A 1·0-mL

sample (in triplicate) was placed in a ultrafiltration tube (MPS-1, micro-partition system, Amicon) and centrifuged at 3000 *g* for 10 min. The ultra-filtrate was then assayed for parent drug and its hydrolysis product by HPLC. To eliminate the possibility of error as a result of hydrolysis, an identical sample was placed in a conventional test tube and subjected to the same procedure. This control group (also in triplicate) was used to adjust for the percentage of spontaneous hydrolysis of the ultrafiltrate under the experimental conditions (7·9, 6·0, 10·6% at a BSA concentration of 2% and 10·6, 8·8, 11·5% at a BSA concentration of 4% for acetylsalicylic acid, *n*-pentanoylsalicylic acid and *n*-hexanoylsalicylic acid, respectively).

The f_u was determined as the ratio of the free concentration to the total concentration of the solute.

In-situ rat-liver perfusion

Mature female Sprague-Dawley rats, 200–250 g, were obtained from the University of Queensland Animal Breeding Facility. Before the experiment the animals were given access to a standard laboratory diet. Rats were anaesthetized by interperitoneal injection of pentobarbital sodium (60 mg kg^{-1}, Boehringer Ingelheim). After laparotomy animals were heparinized (heparin sodium, David Bull Laboratories, Australia; 200 units) via the inferior vena cava. The bile duct was cannulated with PE10 (Clay Adams, NJ). The portal vein was cannulated with a 16G intravenous catheter and the liver was perfused at 30 mL min^{-1}, via this cannula, with 0%, 2% or 4% BSA in Krebs–Henseleit bicarbonate buffer (pH 7·4) which was oxygenated using a silastic tubing lung ventilated with carbogen (95% O_2–5% CO_2). Non-re-circulating perfusion was achieved with a peristaltic pump (Cole-Palmer, IL).

After perfusion the animals were killed by thoracotomy and the thoracic inferior vena cava was cannulated with PE 240 (Clay Adams, NJ). The rats were placed in a temperature-controlled perfusion cabinet at 37°C during the experiment. Liver viability was assessed by oxygen consumption, bile flow, and macroscopic appearance.

Bolus studies

After a 10-min perfusion-stabilization period, a bolus injection (50 μL), containing a saturated solution of *O*-acyl salicylate ester in Krebs–Henseleit buffer was injected into the liver and outlet perfusate was collected for 60 s. Previous experiments had shown that after 60 s at this flow-rate ester concentrations in the perfusate were negligible (Mellick et al unpublished). Nine livers were used in the study. In each liver a maximum of four injections was made with the order of injection randomized and no repeat of the same injection in the same rat. A stabilization period of 10 min was afforded between the changing of perfusate and the next injection. Over the entire study, the hepatic availability (F) of acetylsalicylic, *n*-pentanoylsalicylic or *n*-hexanoylsalicylic acid was determined at perfusate concentrations of 0, 2 and 4% BSA (w/v) with each determination being performed in quadruplicate. The total perfusion time for each liver was less than 2 h.

In perfused rat liver experiments with *n*-pentanoyl- and *n*-hexanoylsalicylic acids, outflow samples were acidified to pH 2·0 by addition of 1 M HCl to prevent ionization and extracted into 2·5 vols ether. The extraction mixture was immediately cooled in the freezer to −20°C, which resulted in the solidification of the aqueous phase. The ether fraction was then removed, reduced to dryness by evaporation in air and reconstituted in 100 μL mobile phase for HPLC analysis. A known concentration of each ester in Krebs buffer was concurrently extracted and assayed with the perfusate samples to assess the extent of spontaneous hydrolysis; results from the perfusate samples were corrected for this. By use of this extraction technique more than 90% of the original ester was recovered as parent ester or salicylic acid.

Influence on f_u value of trace protein produced from rat isolated liver: is f_u of a solute in albumin-free perfusate equal to 1?

Albumin-free Krebs–Henseleit buffer (pH 7·4) was perfused at 30 mL min^{-1} through a rat isolated liver. The outlet perfusate was collected for 60 s after 10-min perfusion-stabilization. A 500-μM solution of each salicylate ester was then prepared in this outlet perfusate. The unbound fraction (f_u) of parent drug and its hydrolysis product (salicylic acid) were investigated using the ultra-filtration method described above.

Determination of hepatic availability, and dispersion model analysis

The hepatic availability (F) of the esters were determined from the equation:

$$F = C_{out}V_{out}/Dose \quad (1)$$

where C_{out} is the outflow ester concentration in the volume (V_{out}) of outflow sample.

According to the dispersion model of hepatic elimination, the hepatic availability (F) of an extracted solute is given by the equation (Roberts et al 1990):

$$F = \exp[(1 - a)/2D_N] \quad (2)$$

where $a = \sqrt{(1 + 4R_N D_N)}$ and D_N is the dispersion number which describes the spread of solute-normalized variance for a non-extracted reference indicator in the liver after bolus input and is equal to half the normalized variance for a non-extracted reference indicator ($CV^2/2$). R_N is the efficiency number which characterizes the elimination of solute by the liver and is defined by the equation:

$$R_N = f_u PCL_{int}/[Q(P + CL_{int})] \quad (3)$$

where f_u is the unbound fraction of solute in the blood, P is the hepatocyte permeability to the solute, CL_{int} is the intrinsic clearance of the liver (defined as $CL_{int} = i = 1 \Sigma n V_{m,i} K_{m,i}$ (Gillette 1971)), and Q is the perfusate flow-rate. Equation 2 was used to determine R_N using F determined from the experimental results and a D_N value of 0·3 determined previously from the outflow curves (n = 16) of non-extracted extracellular reference solute [^{3}H]sucrose in an identical preparation (Mellick et al unpublished). R_N/f_u was then plotted against albumin concentration to determine whether the removal of these solutes by the liver, when corrected for the unbound fraction of solute, remained constant as albumin concentration was increased.

Statistical analysis

All data are presented as mean ± s.d. (n = 4) unless otherwise indicated. Differences between groups of data were assessed by analysis of variance, with $P < 0.05$ being regarded as indicative of significance.

Results

Table 1 lists the unbound fraction (f_u) of the aspirin analogues at various protein concentrations (0, 2 and 4% BSA in Krebs buffer). The strength of protein binding increases in proportion to lipophilicity (*n*-hexanoylsalicylic acid > *n*-pentanoylsalicylic acid > acetylsalicylic acid).

Table 2 lists the hepatic availability (F) data measured for each aspirin analogue at various protein concentrations in bolus studies. For all esters F was shown to decrease with increasing carbon chain length of the *O*-acyl group and to increase with increasing BSA concentration in the perfusate. An order effect was shown by analysis of variance to be insignificant for the F values obtained from identical injections with different rats (data not shown). It is implied that no 'order' effect exists in the injection procedure.

Table 3 lists values of the efficiency number (R_N), a ($=\sqrt{(1 + 4R_N D_N)}$), and R_N/f_u for the esters, determined by the dispersion model of hepatic elimination (equation 2) at various protein concentrations. The R_N of the esters increases with increasing lipophilicity but decreases with increasing albumin concentration in the perfusate. In this homologous series the highest R_N was measured for *n*-hexanoylsalicylic acid at all protein concentrations.

Figure 2 depicts the relationship between the concentration of albumin and R_N/f_u for each solute. The R_N/f_u of *n*-hexanoylsalicylic acid increases significantly as albumin concentration increases from 0 to 2% ($P < 0.05$), from 0 to 4% ($P < 0.001$)

Table 1. Unbound fraction (f_u) for the aspirin analogues at various protein concentrations.

BSA (%)	Unbound fraction (f_u)		
	Acetylsalicylic acid	*n*-Pentanoylsalicylic acid	*n*-Hexanoylsalicylic acid
0	1·01 ± 0·03	0·98 ± 0·03	0·96 ± 0·02
2	0·70 ± 0·06	0·43 ± 0·04	0·23 ± 0·05
4	0·50 ± 0·07	0·28 ± 0·02	0·08 ± 0·01

Data are means ± s.d., n = 4.

Table 2. Hepatic availability (F) data for aspirin analogue bolus studies with different concentrations of protein.

BSA (%)	Hepatic availability (F)		
	Acetylsalicylic acid	*n*-Pentanoylsalicylic acid	*n*-Hexanoylsalicylic acid
0	0·78 ± 0·09	0·26 ± 0·04	0·03 ± 0·01
2	0·94 ± 0·06	0·29 ± 0·02	0·13 ± 0·04
4	0·97 ± 0·02	0·39 ± 0·03	0·18 ± 0·04

Data are means ± s.d., n = 4.

Table 3. Efficiency number (R_N), $\sqrt{(1 + \{4 \times \text{efficiency number} \times \text{dispersion number}\})}$, and (efficiency number)/(unbound fraction*) for aspirin analogue bolus studies with different concentrations of protein.

BSA (%)	Acetylsalicylic acid	*n*-Pentanoylsalicylic acid	*n*-Hexanoylsalicylic acid
Efficiency number			
0	0·27 ± 0·13	1·90 ± 0·28	7·28 ± 1·08
2	0·07 ± 0·06	1·69 ± 0·12	3·36 ± 0·71
4	0·03 ± 0·02	1·21 ± 0·12	2·63 ± 0·46
$\sqrt{(1 + \{4 \times \text{Efficiency number} \times \text{dispersion number}\})}$			
0	1·15 ± 0·07	1·80 ± 0·09	3·09 ± 0·21
2	1·04 ± 0·04	1·73 ± 0·04	2·22 ± 0·19
4	1·02 ± 0·01	1·56 ± 0·04	2·02 ± 0·13
(Efficiency number)/(unbound fraction)			
0	0·20 ± 0·12	1·93 ± 0·34	7·51 ± 1·20
2	0·15 ± 0·10	3·98 ± 0·65	15·58 ± 6·73
4	0·12 ± 0·08	4·35 ± 0·74	33·66 ± 10·09

*Table 1. Data are means ± s.d., n = 4.

and from 2 to 4% ($P < 0·02$). The R_N/f_u of *n*-pentanoylsalicylic acid increases significantly as albumin concentration increases from 0 to 2% ($P < 0·001$) and from 0 to 4% ($P < 0·001$) but not significantly as it increases from 2 to 4%. In contrast, the R_N/f_u of acetylsalicylic acid remained relatively constant as albumin concentration was increased. Figure 2 also shows that the predicted R_N/f_u values for all esters in this study remained constant as albumin concentration was increased and only for acetylsalicylic acid did the experimentally observed data match predicted values.

Discussion

It is generally believed that mechanisms of hepatic uptake of solutes are driven completely by the fraction that is not bound to carrier proteins such as albumin (Gillette & Pang 1977). However, some evidence suggests that the binding protein albumin plays a more complex role in the hepatic uptake of some organic anions. Highly albumin-bound substances, including long-chain free fatty acids (Weisiger et al 1981; Barnhart et al 1983), bilirubin (Scharschmidt et al 1975; Inoue et al 1985), bile acids (Forker & Luxon 1981) and dyes such as bromosulphophthalein (Goresky 1964) and rose bengal (Forker et al 1982; Forker & Luxon 1983; Tsao et al 1988) are removed by the liver from the circulation with outstanding efficiency, despite their strong binding to albumin. For these solutes, increases in albumin concentration seem to enhance hepatic extraction, a phenomenon denoted albumin-mediated transport. We have examined whether this phenomenon exists for a homologous series of salicylate esters in the perfused rat liver.

Table 1 shows that increasing the albumin concentration from 0 to 4% resulted in 50, 72 and 92% reduction in free acetyl-, *n*-pentanoyl- and *n*-hexanoylsalicylic acid, respectively. In comparison, hepatic extraction (1 − F) (Table 2) decreased by 86, 18 and 16%, respectively, for the same esters. Similar results were obtained for *n*-pentanoyl- and *n*-hexanoylsalicylic acids in studies by Forker & Luxon (1981) who showed that a 10-fold increase in albumin results in a 5-fold reduction in free taurocholate concentration, but only a 50% reduction in uptake.

Figure 2 shows that R_N/f_u for acetylsalicylic acid remained relatively constant as albumin concentration was increased. For this solute, hepatic elimination can be explained by a conventional approach. However, R_N/f_u for *n*-pentanoylsalicylic acid increased significantly as albumin concentration increased from 0 to 2% and 4% but not significantly from 2% to 4%. In contrast, R_N/f_u for *n*-hexanoylsalicylic acid was shown to increase significantly as albumin concentration increased from 0 to 2%, 0 to 4% and 2 to 4%. According to the dispersion model of hepatic elimination, P and CL_{int} are independent of albumin concentration. Thus, we can predict R_N/f_u values for the esters at 2 and 4% albumin concentrations on the basis of $PCL_{int}/(P + CL_{int})$ values obtained for each solute using from 0% BSA (equations 2 and 3). The predicted R_N/f_u values for all three esters remained constant as albumin concentration was increased and only for acetylsalicylic acid did the experimentally observed data match the predicted values. Therefore, the conventional approach cannot be used to explain the results for *n*-pentanoyl- and *n*-hexanoylsalicylic acids, implying that the presence

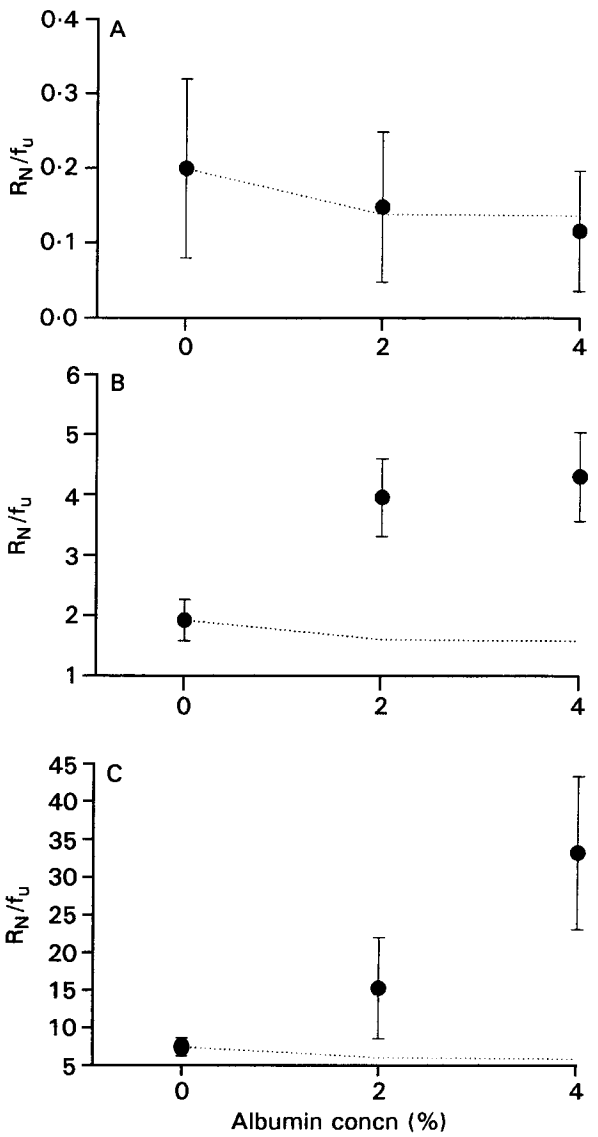

Figure 2. The relationship between the concentration of albumin and R_N/f_u (R_N corrected for the unbound fraction of solute). A. Acetylsalicylic acid; B. *n*-pentanoylsalicylic acid; C. *n*-hexanoylsalicylic acid. ●, Experimentally observed data; ·····, R_N/f_u values predicted for the esters at albumin concentrations of 2 and 4% on the basis of $PCL_{int}/(P+CL_{int})$ values obtained from equations 2 and 3 for each solute for 0% BSA.

of albumin facilitates the uptake of the unbound esters.

The unbound fraction of solute (f_u) in Krebs buffer containing no BSA is generally regarded as 1. However, the isolated liver still produces trace amounts of protein during perfusion (Gores et al 1986). If f_u for 0% BSA is < 1, then the removal efficiency (R_N) calculated for 0% BSA (equation 3) will be an underestimate. This could explain the increase in R_N/f_u observed for *n*-pentanoyl- and *n*-hexanoylsalicylic acids as albumin concentration is increased. Hence, we investigated the influence of this trace amount of protein on f_u. The results of this protein binding study of albumin-free perfusate collected after passage through the liver revealed that the influence of the trace amount of protein produced from the isolated liver is negligible ($f_u = 0{\cdot}99 \pm 0{\cdot}04$, $0{\cdot}99 \pm 0{\cdot}03$ and $0{\cdot}97 \pm 0{\cdot}01$ for acetyl, *n*-pentanoyl- and *n*-hexanoylsalicylic acids, respectively). Thus, there is a real increase of R_N/f_u for *n*-pentanoyl- and *n*-hexanoylsalicylic acids when albumin concentration is increased from 0 to 2% or from 0 to 4%.

The conventional pharmacokinetic view is that the uptake rate is proportional to the unbound concentration of the drug in the perfusate (Gillette & Pang 1977). Therefore, equation 3 is valid and R_N is proportional to f_u at different albumin concentrations such that:

$$R_{N(Y\%)}/R_{N(X\%)} = f_{u(Y\%)}/f_{u(X\%)} \quad (4)$$

where X% and Y% represent the albumin content of the perfusate. However, our work showed that this equation is not always valid. Thus, the conventional parameters (f_u, P, CL_{int}, and Q) are insufficient to explain some of our experimental results, implying that a 'facilitative transport' mechanism is involved in the process of hepatic elimination of *n*-pentanoyl- and *n*-hexanoylsalicylic acids. We can define a symbol 'ϕ' to represent the sum of the extra factors required such that equation 4 becomes:

$$R_{N(Y\%)}/R_{N(X\%)} = (f_{u(Y\%)}/f_{u(X\%)})\phi_{X\to Y} \quad (5)$$

where $\phi_{X\to Y}$ can be thought of as a 'pseudo facilitation factor'. The values of $\phi_{X\to Y}$ for *n*-pentanoyl- and *n*-hexanoylsalicylic acids are greater than unity and increase with increasing protein content of the perfusate (for *n*-pentanoylsalicylic acid $\phi_{X\to Y} = 2{\cdot}08 \pm 0{\cdot}29$ when protein content increases from 0% to 2% and $2{\cdot}26 \pm 0{\cdot}20$ when it increases from 0% to 4%; for *n*-hexanoylsalicylic acid the respective values are $1{\cdot}98 \pm 0{\cdot}29$ and $4{\cdot}40 \pm 0{\cdot}39$).

At present the basis of protein facilitation remains controversial. The unstirred diffusion-layer theory (Bass & Pond 1987) likely to be valid in hepatocyte suspensions (Pond et al 1992) might not necessarily be as evident for perfused liver preparations, for which the unstirred layer would be much thinner. The recent work of Chou et al (1995) suggests that in the perfused liver such a layer might explain the plateau in the relationship between solute permeability and lipophilicity for highly lipophilic solutes. Morgan et al (1990) have argued that albumin facilitation might be an arte-

fact arising from the equilibrium kinetics between the perfusate and hepatocyte.

Conclusions

Use of the in-situ perfused rat liver to examine the effect of changing perfusate protein content on hepatic extraction for *O*-acyl esters of salicylic acid has shown that for the three esters investigated F values decrease with increasing *O*-acyl carbon chain length and increase with increasing albumin concentration in the perfusate. The dispersion-model-derived efficiency number (R_N) of the esters was shown to increase with increasing lipophilicity and decrease with increasing albumin concentration in the perfusate. The unbound fraction (f_u) of the esters was shown to decrease with lipophilicity. R_N/f_u for acetylsalicylic acid remained relatively constant as albumin concentration was increased whereas that for *n*-pentanoyl- and *n*-hexanoyl-salicylic acids increased significantly as albumin concentration was increased from 0% to 4%. Thus, for these more lipophilic solutes the presence of albumin apparently facilitates the uptake of unbound solute relative to acetylsalicylic acid.

Acknowledgements

The authors acknowledge the support of the National Health and Medical Research Council of Australia and the Queensland and New South Wales Lions Kidney and Medical Research Foundation.

References

Barnhart, J. L., Witt, B. L., Hardison, W. G., Berk, R. N. (1983) Uptake of iopanoic acid by isolated rat hepatocytes in primary culture. Am. J. Physiol. 244: G630–G636

Bass, L., Pond, S. M. (1987) The puzzle of rates of cellular uptake of protein-bound ligands. In: Pecile, A., Rescigno, A. (eds), Pharmacokinetics: Mathematical and Statistical Approaches to Metabolism and Distribution of Chemicals and Drugs, Plenum, London, pp 241–265

Chou, C. H., McLachlan, A. J., Rowland, M. (1995) Membrane permeability and lipophilicity in the isolated perfused rat liver: 5-ethylbarbituric acid and other compounds. J. Pharmacol. Exp. Ther. 275: 933–940

Forker, E. L., Luxon, B. A. (1981) Albumin helps mediate removal of taurocholate by rat liver. J. Clin. Invest. 67: 1517–1522

Forker, E. L., Luxon, B. A. (1983) Albumin-mediated transport of rose bengal by perfused rat liver. Kinetics of the reaction at the cell surface. J. Clin. Invest. 72: 1764–1771

Forker, E. L., Luxon, B. A., Snell, M., Shurmantine, W. O. (1982) Effect of albumin binding on the hepatic transport of rose bengal: surface-mediated dissociation on limited capacity. J. Pharmacol. Exp. Ther. 223: 342–347

Gillette, J. R. (1971) Factors affecting drug metabolism. Ann. N. Y. Acad. Sci. 179: 43–61

Gillette, J. R., Pang, K. S. (1977) Theoretical aspects of pharmacokinetic drug interactions. Clin. Pharmacol. Ther. 22: 623–639

Gores, G. J., Kost, L. J., LaRusso, N. F. (1986) The isolated perfused rat liver: conceptual and practical considerations. J. Hepatol. 6: 511–517

Goresky, C. A. (1964) Initial distribution and rate of uptake of sulfobromophthalein in the liver. Am. J. Physiol. 207: 13–26

Hung, D. Y., Mellick, G. D., Prankerd, R. J., Roberts M. S. (1997) Synthesis, identification, characterization, protein binding and stability of ester derivatives of salicylic acid and diflunisal. Int. J. Pharm. 153: 25–39

Inoue, M., Hirata, E., Morino, Y. (1985) The role of albumin in the hepatic transport of bilirubin: studies in mutant analbuminemic rats. J. Biochem. 97: 737–743

Morgan, D. J., Stead, C. K., Smallwood, R. A. (1990) Kinetic assessment of apparent facilitation by albumin of cellular uptake of unbound ligands. J. Pharmacokin. Biopharm. 18: 121–135

Pond, S. M., Davis, C. K. C., Bogoyevitch, M. A., Gordon, R. A., Weisiger, R. A., Bass, L. (1992) Uptake of palmitate by hepatocyte suspensions: facilitation by albumin? Am. J. Physiol. 262: G883–G894

Reed, R. G., Burrington, C. M. (1989) The albumin receptor effect may be due to a surface-induced conformational change in albumin. J. Biol. Chem. 264: 9867–9872

Roberts, M. S., Fraser, S., Wagner, A., McLeod, L. (1990) Residence time distributions of solutes in the perfused rat liver using a dispersion model of hepatic elimination. 1. Effect of changes in perfusate flow and albumin concentration on sucrose and taurocholate. J. Pharmacokin. Biopharm. 18: 209–234

Scharschmidt, B. F., Waggoner, J. G., Berk, P. D. (1975) Hepatic organic anion uptake in the rats. J. Clin. Invest. 56: 1280–1292

Schwab, A. J., Goresky, C. (1991) Free fatty acid uptake by polyethylene: what can one learn from this? Am. J. Physiol. 261: G896–G906

Sluijs, P. V. D., Postema, B., Meijer, D. K. F. (1987) Lactosylation of albumin reduces uptake rate of dibromosulfophthalein in perfused rat liver and dissociation rate from albumin in vitro. Hepatology 7: 688–695

Stremmel, W., Potter, B. J., Berk, P. D. (1983) Studies of albumin binding to rat liver plasma membranes. Implications for the albumin receptor hypothesis. Biochim. Biophys. Acta. 756: 20–27

Tsao, S. C., Sugiyama, Y., Shinmura, K. (1988) Protein-mediated hepatic uptake of rose-bengal in analbuminemic mutant rats (NAR): albumin is not indispensable to the protein-mediated transport of rose-bengal. Drug Metab. Dispos. 16: 482–489

Weisiger, R. A. (1985) Dissociation from albumin: a potentially rate-limiting step in the clearance of substances by the liver. Proc. Natl Acad. Sci. USA 82: 1563–1567

Weisiger, R. A., Ma, W. L. (1987) Uptake of oleate from albumin solutions by rat liver: failure to detect catalysis of the dissociation of oleate from albumin by an albumin receptor. J. Clin. Invest. 79: 1070–1077

Weisiger, R. A., Gollan, J., Ockner, R. (1981) Receptor for albumin on the liver cell surface may mediate uptake of fatty acids and other albumin-bound substances. Science 211: 1048–1051

Weisiger, R. A., Zacks, C. M., Smith, N. D., Boyer, J. L. (1984) Effect of albumin binding on extraction of sulfobromophthalein by perfused elsamobranch liver: evidence for dissociation limited uptake. Hepatology 4: 492–501