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METABOLISM AND BILIARY EXCRETION OF THE LIPOPHILIC DRUG MOLECULES, IMIPRAMINE AND DESMETHYLIMIPRAMINE IN THE RAT—II

UPTAKE INTO BILE MICELLES

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Abstract—Uptake of imipramine and desmethylimipramine, but not of hydrophilic metabolites, into the bile salt-phospholipid-micelles of bile could be demonstrated by equilibrium dialysis and ultracentrifuge sedimentation using rat bile or micellar model systems. Biliary proteins and small molecules which do not form micelles do not participate in uptake. Bile salt–lecithin–imipramine micelles have been calculated from the experiments to have a molecular composition of about 60:10:7 and a molecular weight of 36,000. Possible mechanisms of the concentrative transfer of lipophilic drug molecules into the bile are discussed.

PART I of this paper[1] reports the finding of biliary excretion of the lipophilic drug molecules, imipramine (IP) and desmethylimipramine (DMI). With both rats with bile fistulas and isolated perfused rat livers the bile/plasma concentration ratios of these compounds were in the order of magnitude of 50. This is indicative for a concentrative transfer.

An attempt was made to elucidate the concentrative mechanism of biliary excretion of lipophilic drug molecules. Since endogenous lipids like cholesterol are incorporated into the bile salt–phospholipid micelles of the bile, the hypothesis was tested whether this could also be the case for lipophilic drugs.[2] Hofmann[3] has reported successful micellar solubilizations of lipophilic foreign compounds *in vitro*. Our experiments were done with bile of normal and IP-treated rats as well as with micellar model systems as indicated by Neiderhiser and Roth.[4]

MATERIALS AND METHODS

Animals and drugs

Male Wistar rats of 210–300 g body weight were used. The hydrochlorides of imipramine (IP), desmethylimipramine (desipramine, DMI) and the fumarate of 2-hydroxydesipramine (2-OH-DMI) were generously donated by Geigy Ltd., Basel. Other chemicals used are mentioned below. The collection of bile from canulated rats has been described in part I of this paper.[1]

Analytical methods

Extraction of biological fluids or model solutions has been described in a previous paper.[5] IP or DMI were extracted at pH 10 or 12·5 with *n*-hexane and the optical

density measured at 254 nm. The phenolic metabolites were extracted at pH 10 with 1,2-dichloroethane and measured at 255 nm. A Unicam SP 800 Spectrophotometer was used.

Micelle–drug interaction

Pooled fistula bile from untreated rats was used. For one experiment the bile was obtained after treating the rats with 50 mg/kg IP i.p. For another the bile of untreated rats was predialyzed several times up to 3 days against 2 ml of phosphate buffer in order to remove the small molecules. In six samples of normal rat bile the following values were determined: Bile salts[6] 10–25 mg/ml, phospholipid[7, 8] 1·5–3·9 mg/ml. A cholesterol concentration of 0·13 mg/ml and a pH of 7·8–8·6 are reported in the literature.[9]

These values served as a basis for the preparation of micellar model solutions (Table 1) whereby systems I and III correspond to rat bile. The bile salts used are specified under Results. They were commercial products: Sodium cholate (Fluka, Switzerland), sodium deoxycholate (Merck, Germany), and the mixture of bile salts mentioned in part I.[1] Egg lecithin and cholesterol were recrystallized[10] before use. The following buffer solutions of pH 8·2 and ionic strength 0·17 were used: Tris,[11] phosphate,[12] barbital and borax-phosphate.[13]

Equilibrium dialysis experiments were carried out in a recently developed apparatus described by Weder and Bickel.[14] There are two dialysis cells, one containing 2 ml of micellar solution and the other 2 ml of buffer solution. The drug added to the buffer solution or contained in bile was allowed to dialyze at 20° for 4 hr. This duration had been shown to suffice for concentration equilibrium of IP, DMI and 2-OH-DMI. Experiments with ^{14}C-IP showed that 8–20 per cent of the drug is adsorbed to the membrane. This effect can be neglected, since the drug concentrations after dialysis were measured in both cells.

Sedimentation of micelles was achieved by ultracentrifugation in an MSE Superspeed centrifuge for 4 hr at 120,000 *g* and 20°. Four ml samples of micellar solutions were centrifuged. After ultracentrifugation 1 ml of deep yellow bottom layer and 3 ml of light yellow supernatant were analyzed for drug content. No difference in drug concentration was observed after ultracentrifugation in the absence of micelles.

Using a Spinco E analytical ultracentrifuge, the sedimentation of model system I with deoxycholate and lecithin in phosphate buffer was studied in the presence and in the absence of added IP (1 mg/ml). The two samples were placed in two separate cells and run simultaneously according to Verschure[15] for 3 hr at 200,000 *g* and 20°. The sedimentation diagrams of both cells were recorded.

RESULTS

Bile salt–phospholipid micelles which occur in the bile have an apparent molecular weight of about 30,000 and are therefore not dialyzable. In order to test the feasibility of equilibrium dialysis, micellar model solutions were prepared and dialyzed against buffer. The concentrations of the components of the model system (bile salt and lecithin) were determined in the cell containing the micellar system (c_m) and in the buffer cell (c_b) after equilibrium dialysis. The results are represented in Table 2, which also indicates the concentration ratio c_m/c_b.

Table 3 summarizes the results of experiments in which IP dissolved in various buffer

TABLE 1. MICELLAR MODEL SYSTEMS*

Model system	Bile salt	Lecithin	Cholesterol
I	10·75	2·65	0
III	10·75	2·65	0·48
IV	20·00	0	0

* mg/ml in buffer solution.

TABLE 2. CONCENTRATIONS (mg/ml) OF COMPONENTS OF MICELLAR MODEL SYSTEMS AFTER EQUILIBRIUM DIALYSIS

	Cell containing micellar solution		Buffer cell	
	$t = 0$	$t = 4$ hr c_m	$t = 4$ hr c_b	c_m/c_b
*System I**				
Cholate	10·75	9·0	1·2	7·5
Lecithin	2·65	2·5	0·1	25
*System IV**				
Cholate	20·00	13·9	5·6	2·5

Mean values of 2 experiments. Phosphate buffer.
* See Table 1.

TABLE 3. CONCENTRATIONS OF IMIPRAMINE (3·15 μmoles/ml) AFTER EQUILIBRIUM DIALYSIS AGAINST RAT BILE (μmoles/ml)

Bile	Buffer		Bile cell c_m	Buffer cell c_b	c_m/c_b
Normal	Tris	(2)	2·64	0·36	7·3
Normal	Barbital	(2)	2·71	0·26	10·4
Normal	Borax-phosphate	(2)	2·57	0·39	6·6
Normal	Phosphate	(2)	2·63	0·38	6·9
Predialyzed 12 hr	Phosphate	(1)	2·58	0·38	6·8
Dialysate	Phosphate	(1)	1·49	1·47	1·0
Predialyzed 24 hr	Phosphate	(1)	2·39	0·57	4·2
Dialysate	Phosphate	(1)	1·55	1·41	1·1
Predialyzed 72 hr	Phosphate	(1)	2·34	0·60	3·9

In brackets number of experiments.

solutions was dialyzed against bile from normal rats. IP becomes highly concentrated in the bile cell even if the bile has been predialyzed for 12 hr or more. With the dialysate, however, no concentrative effect is observed.

In another experiment IP and its metabolites contained in the fistula bile of an IP-treated rat were allowed to dialyze against buffer. Table 4 shows the pronounced differences in the c_m/c_b ratios between IP and lipophilic metabolites (DMI, DDMI) on one hand and hydrophilic metabolites on the other.

IP dissolved in buffer was dialyzed against various micellar model systems. The results of this series are summarized in Table 5 which again shows a concentrative

TABLE 4. CONCENTRATIONS OF IMIPRAMINE METABOLITES OF THE BILE OF A RAT TREATED WITH IMIPRAMINE AFTER EQUILIBRIUM DIALYSIS (μmoles/ml)

	Bile cell c_m	Buffer cell c_b	c_m/c_b
Lipophilic metabolites*	0·045	0·015	3·0
Hydrophilic metabolites†	0·310	0·235	1·3

One experiment. Phosphate buffer.
* IP, DMI, DDMI, calculated as IP.
† 2-hydroxy metabolites and glucuronides calculated as 2-OH-DMI.

TABLE 5. CONCENTRATIONS OF IMIPRAMINE (3·15 μmoles/ml) AFTER EQUILIBRIUM DIALYSIS AGAINST MICELLAR MODEL SYSTEMS (μmoles/ml)

System	Bile salt*	Duration (hr)	Micellar system cell c_m	Buffer cell c_b	c_m/c_b
I	cholate	1	2·31	0·44	5·2
I	cholate	4	2·39	0·36	6·7
I	cholate	10	2·29	0·52	4·4
I	deoxycholate	1	2·50	0·27	9·3
I	deoxycholate	4	2·56	0·23	11·1
I	mixture†	1	2·29	0·39	5·9
I	mixture†	4	2·35	0·27	8·7
III	cholate	4	2·35	0·44	5·3
III	mixture†	4	2·50	0·32	7·8
IV	cholate	4	2·08	0·99	2·1
IV	mixture†	4	2·29	0·79	2·9

Means of 2 experiments. Tris buffer.
* see Table 1,
† see Ref. 1.

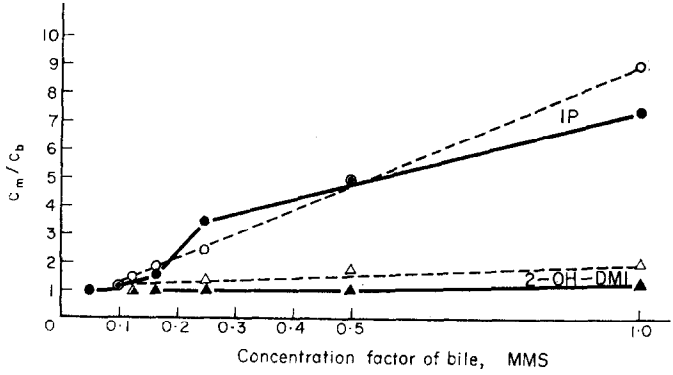

FIG. 1. Effect of diluting bile or micellar model system (MMS) on concentration ratio c_m/c_b. 3·15 μmoles/ml IP or 2·50 μmoles/ml 2-OH-DMI in Tris buffer; equilibrium dialysis, 4 hr, against (diluted) bile or MMS I with cholate. Bile: ● IP, ▲ 2-OH-DMI. MMS: ○ IP, △ 2-OH-DMI. Means of three experiments.

effect of IP in the micellar compartment. With pure bile salt micelles, i.e. in the absence of phospholipid, the c_m/c_b ratios are significantly lower.

The lipophilic metabolite, DMI, dialyzed against model system I with cholate shows a 5·5-fold concentration ratio in the micellar compartment. With IP dialyzed against the same system and buffer solutions of pH 6·5, 8·2 and 9·0 the following c_m/c_b ratios were determined: 4·3, 6·7 and 9·0 respectively.

Figure 1 represents the decrease in c_m/c_b ratios when IP is dialyzed against increasing dilutions of bile or micellar model systems. A critical micellar concentration is obtained at a dilution of 1:5 to 1:10, equalling a bile salt concentration range of 1–5 mg/ml. In contrast, virtually no interaction with micelles can be observed with the hydrophilic metabolite, 2-OH-DMI.

Ultracentrifugation experiments of IP dissolved in bile and micellar model solutions respectively are represented in Table 6. In this series c_m/c_b is the ratio of the IP concentrations in the micelle-enriched bottom layer and in the supernatant.

TABLE 6. CONCENTRATIONS OF IMIPRAMINE AFTER ULTRACENTRIFUGATION IN RAT BILE OR MICELLAR MODEL SYSTEMS (μmoles/ml)

System*	Bile salt	Initial conc.	Bottom layer c_m	Supernatant c_b	c_m/c_b
Bile		1·58	3·07	0·64	4·8
Bile 1:10		1·58	1·59	1·32	1·2
I	cholate	3·16	5·48	0·96	5·7
I	deoxycholate	3·16	5·34	1·08	4·9
I	mixture†	3·16	5·25	1·14	4·6

Means of 2 experiments. Phosphate buffer.
* See Table 1.
† See Ref. 1.

Finally the sedimentation of the micellar model system I with deoxycholate was studied both in the absence and in the presence of IP in an analytical ultracentrifuge. Identical sedimentation diagrams are obtained for both solutions. An approximate sedimentation constant $s = 1{\cdot}1 \times 10^{-13}$ sec was determined.

DISCUSSION

Endogenous lipophilic compounds like cholesterol[16–19] and sterols[20] are present in the micelles of the bile. With these compounds and even with a lipophilic foreign compound like azobenzene,[3] micellar solubilization can be achieved with synthetic micellar model systems. Micelles are polymolecular aggregates which behave like macromolecules with respect to osmotic activity, dialysis, or sedimentation. Bile salts alone form micelles which, however, are stabilized by phospholipid. These amphiphilic compounds then are arranged so as to form an ionic surface and an inside like a lipid droplet which is able to dissolve truly lipophilic compounds.

This situation suggests that lipophilic drugs which are concentratively transferred into the bile are incorporated in bile micelles. Indeed, IP in various buffer solutions dialyzed against rat bile shows a 7–10-fold concentration in the bile cell (Table 3). Since the possibility exists that during equilibrium dialysis micelles are broken up due to disappearance of free components, the determined concentration ratios are rather

underestimated. The same concentration ratios obtained by dialysis of IP against predialyzed bile, and the ratio of 1·0 by dialysis against bile dialysate rule out a binding to small molecular components of the bile. Inversed dialysis of bile of an IP-treated animal against buffer shows a concentration ratio of about 3 for IP in the rat (Table 4) and in the dog.[21] In contrast, there are near-equal concentrations of the hydrophilic metabolites in both cells.

The substitution of micellar model solutions for bile leads to comparable concentration ratios for IP (Table 5) and also for DMI. The considerable decrease in the ratio by the omission of phospholipid (system IV) demonstrates the influence of this micellar component on lipid uptake which has also been shown to apply for cholesterol uptake.[20]

Dilution experiments with bile and micellar model systems (Fig. 1) demonstrate the decrease of IP uptake with decreasing micelle content and show the approximate critical micellar concentration. The shape of the curve makes binding to bile proteins unlikely. Besides, bile protein concentration is about 100 times lower than in plasma.[16] Finally, the dilution experiments rule out any appreciable micellar uptake of the hydrophilic[22] metabolite, 2-OH-DMI.

The micellar uptake of IP by bile and micellar model solutions shown by dialysis experiments has been confirmed by ultracentrifuge sedimentation (Table 6). The concentration ratios of about 5 are definitely underestimates since the bottom layer and the supernatant do not represent pure micellar and molecular solutions respectively.

The sedimentation constant of a deoxycholate–lecithin micellar system determined in an analytical ultracentrifuge was comparable to the value determined by Verschure *et al.*[15] and identical in the presence and absence of incorporated IP. This compound thus does not constitute the bulk of the micelle. In dialysis experiments with the cholate-lecithin-IP system the non-dialyzable concentrations of the components are 18·1, 3·0 and 2·1 mM. This would correspond to micelles of 60, 10 and 7 molecules of the components and a molecular weight of 36,000. Similar values were obtained for the bile salt–phospholipid–cholesterol micelles of human bile by Nakayama.[17] The cholesterol content of rat bile is at least ten times lower.[9]

The demonstration that lipophilic drugs like IP or DMI are taken up into bile micelles and transported mainly as such does not yet give a satisfactory explanation of the mechanism of concentrative transfer. The basic lack of knowledge concerns the site of micelle formation. If it occurs only in peripheral bile,[15] then an active transport must be assumed. This may apply to either IP-molecules, or, in analogy to quaternary ammonium cations,[23] to protonated IP cations. On the other hand bile micelles may be formed in the liver cell.[20,24] This is suggested by the fact that only micelle-forming lipids are selected for biliary excretion and furthermore by the morphological relationship of canalicular villi to Golgi complexes,[25] or by the excretion of casein micelles by the cells of the mammary gland. In this case intracellular passive accumulation of IP in the micelles would give an erroneous impression of active transfer.[2]

In the three-compartment-system, plasma–liver–bile,[1] IP is rapidly taken up and bound in the liver cell. In the first half hour of IP-treated fistula rats a concentration ratio plasma–liver–bile of 1:360:130 is observed for IP.

However, the concentration gradient liver → bile cannot be the cause for the extent of IP-transfer into bile since the liver → plasma gradient is higher. Furthermore, after

4–5 hr a concentration ratio of about 1:15:45 is observed. Thus IP is then transferred into bile even against a liver → bile gradient. Finally, ductular absorption or secretion can be involved in biliary excretion of endogenous[26] or exogenous[27] compounds. Due to the pH-gradient 8·2:7·4 between rat bile and plasma, absorption of the basic IP is much more likely than secretion. The actual concentrative transfer of IP would therefore be even more pronounced than has been determined by measurements in peripheral bile. The final answer to the origin of bile micelles is likely to await the access to canalicular bile.

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