Biochemical Pharmacology, Vol. 29, pp. 2573–2576.

0006-2952/80/1001–2573 $02.00/0

TRANSPORT RATES OF HEPATIC UPTAKE AND BILIARY EXCRETION OF AN ORGANIC CATION, ACETYL PROCAINAMIDE ETHOBROMIDE

HIROKO NAKAE,*‡ KANJI TAKADA,* SHOZO ASADA* and SHOZO MURANISHI†

*Kobe Women's College of Pharmacy, Higashinada-ku, Kobe 658, and †Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606, Japan

(*Received* 26 *November* 1979; *accepted* 9 *April* 1980)

Abstract—Rates of hepatic uptake and biliary excretion, as well as the T_{max}, of acetyl procainamide ethobromide (APAEB), an organic cation, were estimated in rats. The relationship between hepatic uptake and dose followed saturation kinetics. From the effect of isopropamide iodide on the uptake rate of APAEB and on the binding of APAEB to liver tissues, the saturated uptake was presumed to occur by membrane transport. The excretion rate versus the liver level, however, did not follow saturation kinetics clearly up to T_{max}, whereas the excretory step is considered an active process. The calculated V_{max} for the excretory step was far greater than the maximal uptake rate and T_{max} estimated by constant infusion of APAEB, indicating that the rate-determining step in the biliary excretion of APAEB is not the excretory but the uptake step which is in contrast with that observed with many organic anions.

It is well known that biliary excretion of many organic compounds comprises at least two membrane transport steps, uptake from blood into the hepatocytes and transport from the hepatocytes into bile. A number of studies of hepatic uptake of organic anions in several experimental systems, e.g. isolated liver cells [1–4] and the multiple-indicator dilution technique [5–7] have shown that bile acid and sulfobromophthalein uptake processes are saturable and carrier-mediated [1–7]. The maximum uptake rates of the drugs, compared with the transport maxima, were several times greater, indicating that the uptake process is not rate-limiting in the hepato-biliary transport of these compounds. This is consistent with reports that the rate-limiting step in biliary excretion of organic anions is transport from the hepatocytes into bile [8–10].

It is not clear, however, what mechanism is operative in hepatic uptake and which step is rate-determining in the hepato-biliary transport of organic cations. In the present study, rates of hepatic uptake and biliary excretion of acetyl procainamide ethobromide (APAEB), a non-metabolizable organic cation, were estimated in rats to determine the characteristics of the hepato-biliary transport of the organic cation.

MATERIALS AND METHODS

Chemicals. APAEB was synthesized in our laboratory according to the method of Hwang and Solomon [11]. Isopropamide iodide was provided by the Sumitomo Chemical Co., Ltd., Osaka, Japan.

Animal experiments. Male Wistar rats weighing 220–270 g were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). After laparotomy the renal pedicles were ligated.

In the experiments to estimate the biliary excretion rate, the bile duct was cannulated with polyethylene tubing. APAEB was given via the femoral vein by constant infusion (50 μl/min) or bolus injection. Constant infusion was preceded by a bolus injection equal to 30 min of infusion except at the rate of 70 nmoles/(min · 100 g rat) when the bolus injection was equal to 15 min of infusion. Bile samples were collected at 15-min intervals. To measure the blood level of APAEB during constant infusion, blood samples were obtained via the carotid artery using another group of rats from which the bile samples were collected, but under the same experimental conditions as above. At the end of the experiment, blood was taken from the abdominal aorta and the liver was removed. Rectal temperature was maintained at 37 ± 1° throughout.

The hepatic uptake rate was estimated according to the method of Paumgartner [12]. Exactly 3 min after injection of APAEB via the femoral vein, the blood was taken from the abdominal aorta and the liver was removed. Calculation of the uptake rate was performed from the concentration in liver (C_{liver}, C_l). To eliminate the contribution of the hepatic blood content to the liver level of APAEB, the following formula was used:

$$v = \frac{C_l - (Cp \times 0.18)}{3 \times 0.82},$$

where v represents the amount of APAEB taken up by each g liver/min and Cp the concentration of APAEB in plasma.

Determination of APAEB. The concentration of APAEB in blood, liver and bile samples was determined according to the method reported before [13].

Binding to liver homogenate. A 40% liver homogenate was prepared with 0.15 M KCl solution. After equilibrium dialysis of 5 ml of APAEB solution against 2 ml of the homogenate for 30 hr at 4° with

‡ Author to whom correspondence should be addressed.

Visking cellulose tubing (Union Carbide Corp., New York, NY), the concentration of APAEB in the drug solution was determined. The amount of APAEB bound to the homogenate was calculated from the decrease in concentration in the drug solution.

Data analysis. The parameters of the Michaelis–Menten equation were determined by least squares parameter estimation of non-linear equations using a program provided by M. Arakawa, Hiroshima University, Hiroshima, Japan.

RESULTS

Transport maximum of APAEB. Figure 1 shows rat biliary excretion of APAEB during constant infusion of APAEB at various concentrations. Infusion of 15 nmoles/(min · 100 g rat) progressively raised the excretion rate so that at 120 min it was 8 nmoles/(min · 100 g rat), while the blood level remained constant. During infusion of 50 and 70 nmoles/(min · 100 g), the biliary excretion of APAEB gradually increased but attained a plateau after 120 min. Blood levels of APAEB increased linearly during the experiment. The plateau level for biliary excretion was 24 nmoles/(min · 100 g rat); increasing the infusion rate from 50 nmoles/(min · 100 g rat) to 70 nmoles/(min · 100 g rat) did not increase it. On the basis of these results the maximum excretory rate by this procedure was about 24 nmoles/(min · 100 g rat).

Vonk *et al.* [14], however, reported that the maximum transport rate of APAEB was 111 nmoles/(min · kg), which was obtained after a single bolus injection of 35.3 μmoles/kg of APAEB to rats. Therefore, the biliary excretion of APAEB after a single injection was also investigated in our laboratory. Figure 2 shows representative examples of the excretion rate after injection of 20 and 25 μmoles/300 g rat. The peak level after both doses was about 15 nmoles/(min · 100 g rat), which is less than the maximum rate with constant infusion and was similar to the result by Vonk *et al.* This finding shows that the transport maximum (T_{max}) of APAEB was 24 nmoles/(min · 100 g rat) when obtained by constant infusion, and that the single injection method did not give the correct value of T_{max} for APAEB.

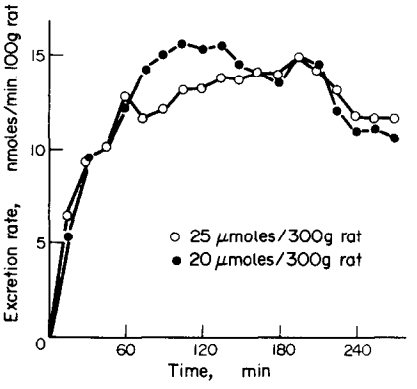

Fig. 2. Biliary excretion of APAEB after a single injection. Representative data of three experiments are plotted.

Hepatic uptake. The hepatic uptake rate of APAEB was determined by the method of Paumgartner [12]; the relation between uptake and dose is shown in Fig. 3. When the dose was increased from 0.33 to 6.7 μmoles/100 g rat, the relationship between uptake rate and dose seems to have followed saturation kinetics, which is consistent with the Michaelis–Menten relation observed with many organic anions. The calculated V_{max} and K_m were 13.4 nmoles/(min · g liver) and 0.47 μmoles/100 g rat respectively.

To further study the saturable uptake process of APAEB, the effects of isopropamide iodide, an organic cation, on hepatic uptake of APAEB and on its binding to liver tissues were investigated. When isopropamide iodide (0.1 μmole/100 g rat) was injected simultaneously with APAEB, the hepatic uptake rate of APAEB was markedly depressed (Fig. 4a). On the other hand, the amount of APAEB bound to liver homogenate was not influenced by the presence of 100 nmoles/ml of isopropamide iodide (Fig. 4b). These findings (that isopropamide iodide inhibited the uptake of APAEB but did not affect the binding to liver tissues) indicate that the saturable hepatic uptake of APAEB was due not to its binding to liver tissue but to a membrane transport system. Therefore, the Michaelis–Menten parameters obtained above are considered to be those

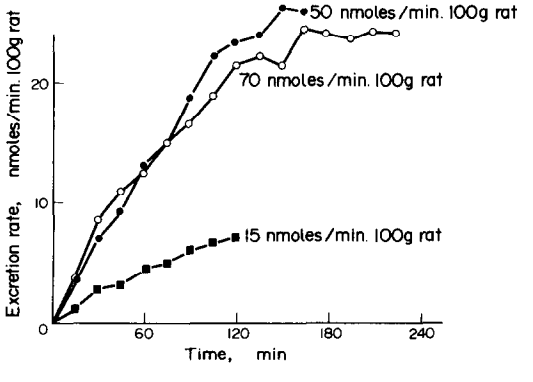

Fig. 1. Biliary excretion of APAEB during constant infusion. Representative data of more than three experiments are plotted.

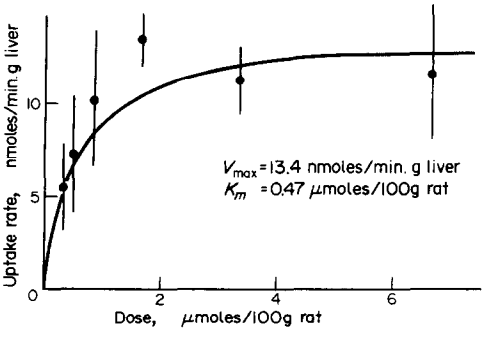

Fig. 3. Relationship between dose and hepatic uptake of APAEB. Each value is the mean ± S.D. of more than five experiments.

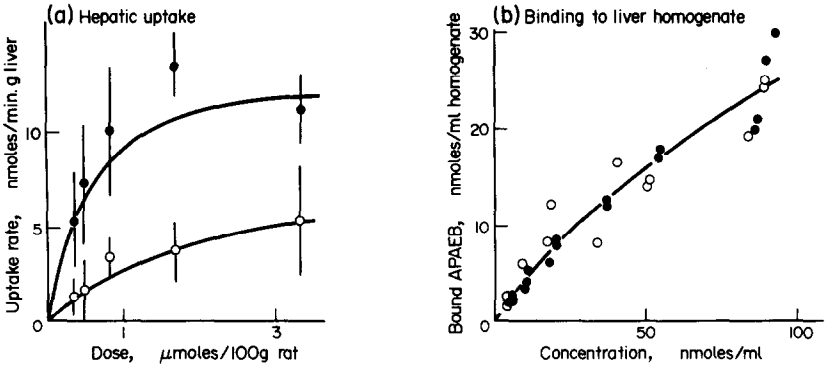

Fig. 4. Effect of isopropamide iodide on hepatic uptake and binding of APAEB. Panel a: (●) control; and (○) isopropamide iodide (0.1 μmole/100 g rat) was administered. Each value is the mean ± S.D. of more than five experiments. Panel b: (●) control; and (○) isopropamide iodide (100 nmoles/ml) was added.

of membrane transport from blood into hepatocytes.

Biliary excretion. Biliary excretion of a number of chemicals from hepatocytes into bile is thought to occur by active transport. To examine the transport parameters of this process, the excretion rate of APAEB was plotted against its concentration in liver at the end of the T_{max} experiment, according to the method of Paumgartner. As shown in Fig. 5, the excretion rate increased almost linearly up to the T_{max} as the liver concentration of APAEB rose. Although saturation kinetics was not observed in this case, the data points were fitted to the Michaelis–Menten equation. The concentration of APAEB in bile was much higher than that in liver, suggesting that this transport process must be an active one. The calculated values of V_{max} and K_m were 126 nmoles/(min · 100 g rat) and 3210 nmoles/g liver respectively. As the T_{max} of APAEB was only 24 nmoles/(min · 100 g rat), the calculated V_{max} was about five times greater than T_{max}. From this finding, the excretory step from hepatocytes into bile does not seem to be rate-limiting in the hepato-biliary transport of APAEB.

DISCUSSION

Although the kinetics of hepato-biliary transport of organic cations have not been investigated extensively, examination of the hepatic uptake rate of APAEB in the present study showed that it followed saturation kinetics in the same manner as many organic anions. Eaton and Klaassen [15] have reported that uptake of another organic cation, procaine amide ethobromide (PAEB), is also saturable by isolated liver cells. But, the V_{max} for hepatic uptake of APAEB was fairly small when compared with those of organic anions previously reported [16–18], one example of which is shown in Table 1 using the data for indocyanine green as estimated by Paumgartner [12]. The mechanism of this uptake process is presumed to be carrier-mediated membrane transport, from the effect that isopropamide iodide has on the hepatic uptake and binding of APAEB to liver tissues. In a previous report, we showed that isopropamide iodide markedly inhibited the biliary excretion of PAEB, and both the liver/plasma and the bile/liver concentration ratios of PAEB were strikingly lowered by the drug [13]. This study demonstrated that inhibition of the membrane transport of PAEB from blood to hepatocytes by isopropamide iodide results in a depression of the liver/plasma concentration ratio of PAEB.

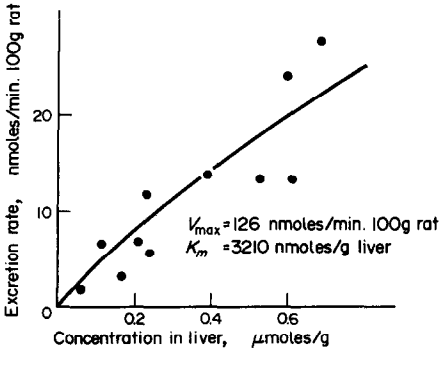

Fig. 5. Relationship between concentration in liver and biliary excretion of APAEB. Each value is the result of one experiment.

The biliary excretory step for organic anions such as indocyanine green and sulfobromophthalein has also been shown to be a saturable process, from the relationship between the excretion rate and its concentration in the liver [12, 19, 20]. As shown in Table 1, the V_{max} for the excretory step of indocyanine green agreed well with the T_{max} and was far smaller than the V_{max} for hepatic uptake. As indocyanine green is not metabolized in the liver, this must indicate that the excretory step is rate-determining in its biliary excretion. For this type of hepato-biliary transport, the V_{max} for the excretory step can be estimated by measuring the T_{max}. In the case of APAEB, however, a clear saturation kinetics was not observed up to T_{max}. This fact can be explained by assuming that the liver concentration could not rise high enough to show the saturation phenomenon because of the small uptake rate of APAEB. The calculated V_{max} for the excretory step of APAEB was far larger than that for the hepatic uptake as shown in Table 1 in support of the assumption. From these results, the rate-determining step in the biliary excretion of APAEB is considered to be the hepatic

Table 1. Hepato-biliary transport parameters of APAEB and indocyanine green

	Hepatic uptake			Biliary excretion	
	T_{max} [nmoles/(min · 100 g rat)]	V_{max} [nmoles/(min · g liver)]	K_m (nmoles/100 g rat)	V_{max} [nmoles/(min · 100 g rat)]	K_m (nmoles/g liver)
APAEB	24	13.4	470	126	3210
Indocyanine green*	21	84.4	570	24.4	250

* Data from Ref. 12.

uptake process, in contrast to the rate-limiting step for many organic anions. This conclusion seems consistent with the report by Klaassen [21] that clearance of PAEB is more dependent on hepatic mass than on interruption of the transfer from liver to bile. For this type of transport, the V_{max} of the excretory step does not agree with the T_{max}.

If hepatic removal is the only route for transfer of the drug, the maximum excretion rate will be the same either by constant infusion or by single injection of the drug. Although this has been shown to be the case for a number of organic anions, the T_{max} of APAEB was obtained only by the constant infusion method. Because plasma levels of APAEB after 90 min were 137 ± 16 nmoles/ml after the bolus injection of 20 μmoles/300 g rat and were 198 ± 23 nmoles/ml during constant infusion of 70 nmoles/(min · 100 g rat), the difference in the maximal excretion rate by the two methods may be due to the difference in the hepatic uptake rate. Therefore, distribution of APAEB to other organs should be taken into consideration to explain the difference. Investigation of this point is now in progress.

Acknowledgements—We are grateful for the technical assistance of Ms. Y. Fujita, Ms. K. Kamino, Ms. N. Hamada, Ms. K. Masuda and Ms. H. Minoshima.

REFERENCES

1. L. R. Schwarz, R. Burr, M. Schwenk, E. Pfaff and H. Greim, *Eur. J. Biochem.* **55**, 617 (1975).
2. M. S. Anwer, R. Kroker and D. Hegner, *Hoppe-Seyler's Z. physiol. Chem.* **357**, 1477 (1976).
3. Y. Laperche, C. Graillot, J. Arondel and P. Berthelot, *Biochem. Pharmac.* **28**, 2065 (1979).
4. C. F. A. van Bezooijen, T. Grell and D. L. Knook, *Biochem. biophys. Res. Commun.* **69**, 354 (1976).
5. J. C. Glasinovic, M. Dumont, M. Duval and S. Erlinger, *J. clin. Invest.* **55**, 419 (1975).
6. J. C. Glasinovic, M. Dumont, M. Duval and S. Erlinger, *Gastroenterology* **69**, 973 (1975).
7. C. A. Goresky, *Am. J. Physiol.* **207**, 13 (1964).
8. A. A. Moss and S. Riegelman, *Abstr. Acad. pharmaceutical Sci.* **5** (No. 2), 118 (1975).
9. H. O. Wheeler, *Archs. intern. Med.* **130**, 533 (1972).
10. I. Sperber, *Pharmac. Rev.* **11**, 109 (1959).
11. S. W. Hwang and H. M. Solomon, *Xenobiotica* **1**, 265 (1971).
12. G. Paumgartner, *Schweiz. med. Wschr.* **105**, Suppl. 1 (1975).
13. H. Nakae, R. Sakata and S. Muranishi, *Chem. pharm. Bull., Tokyo* **24**, 886 (1976).
14. R. J. Vonk, E. Scholtens, G. T. P. Keulemans and D. K. F. Meijer, *Naunyn-Schmiedeberg's Archs. Pharmac.* **302**, 1 (1978).
15. D. L. Eaton and C. D. Klaassen, *J. Pharmac. exp. Ther.* **206**, 595 (1978).
16. J. Reichen and G. Paumgartner, *Am. J. Physiol.* **231**, 734 (1976).
17. A. Dietmaier, R. Gasser, J. Graf and M. Paterlick, *Biochim. biophys. Acta* **443**, 81 (1976).
18. L. F. Rikkers and F. G. Moody, *Gastroenterology* **67**, 691 (1974).
19. R. Clarenburg and C. C. Kao, *Am. J. Physiol.* **225**, 192 (1973).
20. E. L. Forker and G. Gibson, *The Liver, Quantitative Aspects of Structure and Function*, p. 326. Karger, Basel (1973).
21. C. D. Klaassen, *J. Pharmac. exp. Ther.* **191**, 25 (1974).