Biochemical Pharmacology, Vol. 30, No. 11, pp. 1333–1336, 1981.
Printed in Great Britain.

0006-2952/81/111333-04 $02.00/0

TWO SYSTEMS ARE INVOLVED IN THE SULFOBROMOPHTHALEIN UPTAKE BY RAT LIVER CELLS: ONE IS SHARED WITH BILE SALTS

Y. LAPERCHE*, A. M. PREAUX and P. BERTHELOT
Unité INSERM U-99, Hôpital Henri Mondor, 94010 Créteil, France

(*Received* 7 *November* 1980; *accepted* 12 *January* 1981)

Abstract—It is generally believed that the uptake of sulfobromophthalein by rat liver cells is mediated by a single carrier. Accordingly, kinetic plots obtained using a wide concentration range, failed to give any evidence of heterogeneity in the uptake and gave a K_m of 8.9 ± 2.5 μM and a maximal velocity of 4.2 ± 0.8 nmoles/min/10^6 cells. Na^+-taurocholate inhibited competitively, but only partially, sulfobromophthalein uptake by isolated rat liver cells. The taurocholate sensitive component of sulfobromophthalein uptake followed Michaelis Menten kinetics with a K_m of 0.9 μM and a V_{max} of 0.47 nmole/min/10^6 cells. Taurocholate inhibition reveals an heterogeneity in the uptake of sulfobromophthalein not shown in classical kinetic plots, indicating that two carriers are involved in the uptake of this dye by rat liver cells. The taurocholate sensitive carrier which displays an affinity for sulfobromophthalein ten times higher than that of taurocholate insensitive one, is probably identical to the Na^+-independent carrier involved in bile salt uptake.

Studies on the uptake of organic anions, such as sulfobromophthalein (BSP) and bilirubin, by rat liver, carried out either in the whole animal [1], in isolated perfused liver [2] or in isolated cells [3], have shown that this uptake is carrier-mediated and apparently not inhibited by bile salts. It was therefore concluded that organic anions and bile salts enter the liver *via* separate carriers. Recently, however, it has been reported that BSP and bilirubin competitively inhibit the Na^+-independent uptake of bile salts in rat liver cells [4], suggesting that there is a common carrier for the hepatic uptake of BSP, bilirubin and bile salts. Such an hypothesis is conflicting with the absence of effect of bile salts on BSP and bilirubin uptake. This apparent discrepancy may be due to the fact that more than one carrier may be involved in BSP or bilirubin uptake by liver cells, as it has been proposed for the canalicular excretion [5]. This inhibition by bile salts may not have been detected to date due to the choice of substrate concentrations used. By determining BSP uptake in a wide range of concentrations in the present study, we have been able to show that this uptake can be divided into two components; one is competitively inhibited by taurocholate which is the main bile salt found in the rat. This suggests that the uptake of bile salts and anionic drugs by the liver cells can be partially shared.

MATERIAL AND METHODS

Chemicals. [^{35}S]Sulfobromophthalein sodium, purchased from Amersham radiochemical Centre, has a specific activity of 50 mCi/mmole. The counting medium (Ready Solve E) was obtained from Beckman (Irvine CA, U.S.A.) and unlabeled BSP from Fluka (AC Buchs, Switzerland); Na^+-taurocholate was from Maybridge (Tintagel N.C., U.K.). All the other reagents were of analytical grade.

Isolation of liver cells. Hepatocytes were isolated from male Sprague Dawley rats, weighing 250–300 g, maintained on standard chow and having free access to water until killed, as previously described [6]. The cells were washed twice in the incubation medium (121 mM NaCl, 4.9 mM KCl, 1.2 mM $MgSO_4$, 0.13 mM $CaCl_2$, 1.2 mM KH_2PO_4, 16.5 mM Na_2HPO_4) at pH 7.4 and gassed with a mixture of O_2 (95%) and CO_2 (5%). The cell suspension was stored at +4°C in a culture flask at a concentration of about 20.10^6 cells/ml until used. Cell preparations were only used for uptake experiments when 85–95% of cells had a highly refractive membrane under the light microscope and when the intracellular K^+ concentration, determined as previously indicated [6] was higher than 110 mM. We observed no significant change in these values, when the cells were stored as indicated above, for up to 3 hr.

Uptake experiments. Incubations were performed on the cell suspension diluted to 0.5 × 10^6 cells/ml, at 26°C in 8.2 ml of the medium described above. Uptake was initiated by adding labeled BSP (specific activity at arrival: 50 mCi/mmole) dissolved in the same medium. For BSP concentrations higher than 1 μM labeled BSP was diluted four times with unlabeled BSP. The phosphate buffered medium (8.2 ml in a 50 ml erlenmeyer flask) was aerated in open air; the pH remained unchanged during the short time of the assay which never exceeded six minutes, including the preincubation. Taurocholate, when used, was added immediately before BSP in 0.2 ml. Uptake was stopped by dilution of 0.8 ml of incubation medium in 7.2 ml of ice cold medium followed by filtration on glass fiber filters. The filters were

* Author to whom correspondence should be sent.

washed with 7.2 ml of ice cold medium and directly counted using an Intertechnique spectrometer. The initial velocity of the uptake was determined from the slope of the linear portion of the time course curve. Measurements were made in duplicate at 10, 20, 30 and 40 sec, which all agreed within ±5%.

RESULTS

The relationship between extracellular BSP concentration and initial uptake velocity follows Michaelis Menten kinetics as previously shown [1–3]. Even using a wide range of BSP concentrations (0.03–20 μM), we failed to observe any evidence of heterogeneity in the BSP uptake. The Eadie–Hofstee kinetic plot of the data (Fig. 1) gave a straight line from which a K_m of 6.5 μM and a V_{max} of 3.3 nmoles/min/10^6 cells could be calculated. The mean values obtained from five experiments were 8.9 ± 2.5 μM for the K_m and 4.2 ± 0.8 nmoles/min/10^6 cells for the V_{max}. The uptake of BSP by rat liver cells appeared to consist of only a single component; at most, in two experiments, the beginning of a second slope was obtained when BSP concentrations were lower than 0.1 μM.

Na^+-taurocholate partially inhibited the initial velocity of BSP uptake by rat liver cells (Fig. 2). Total inhibition of the initial uptake could not be obtained whatever the concentration of BSP or taurocholate used. The asymptotic values of the curves indicate the portion of BSP uptake subject to taurocholate inhibition. This can be easily determined by the method of Inui and Christensen [7] in which the reciprocal of the decrease in velocity caused by taurocholate inhibition was plotted against the reciprocal of taurocholate concentration. From this plot (Fig. 3) the reciprocal of the rate of BSP uptake subject to inhibition at infinite taurocholate concentration, can be calculated from the intercept with y axis. It appears that the taurocholate sensitive component of BSP uptake can vary from 35 to 80% of the total uptake, in relation with BSP concentration.

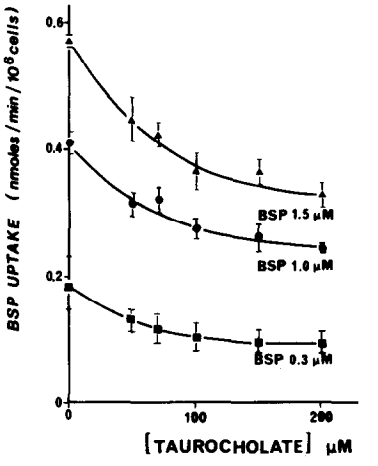

Fig. 2. Na^+-taurocholate effect on the initial velocity of BSP uptake. The curves were obtained at three different BSP concentrations. Each point is the mean (±SEM) of 3 or 4 experiments.

As revealed by the Dixon plot of the data (Fig. 4), taurocholate inhibition of the taurocholate sensitive component of BSP uptake is competitive, with a K_i of 50 μM. Incubation of the cells with 200 μM of taurocholate had no effect on intracellular K^+ content. The taurocholate sensitive component of BSP uptake, determined as indicated in Fig. 3, follows Michaelis Menten kinetics yielding a K_m of 0.9 μM and a V_{max} of 0.47 nmole/min/10^6 cells as calculated from Fig. 5.

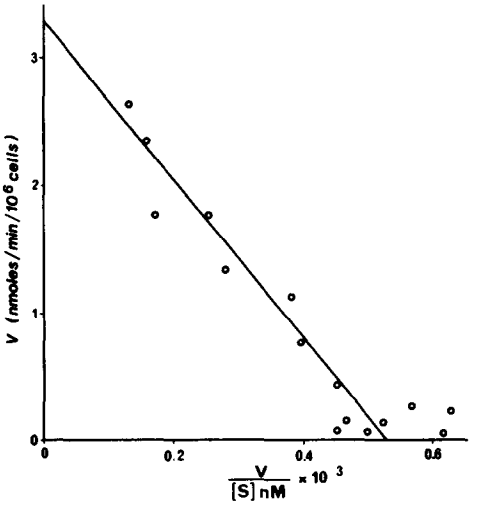

Fig. 1. Eadie-Hofstee plot of BSP uptake by isolated rat liver cells. Initial velocities were determined as indicated under material and methods. From the intercepts on the axis we calculated a V_{max} of 3.3 nmoles/min/10^6 cells and a K_m of 6.5 μM.

DISCUSSION

Partial inhibition of BSP uptake by taurocholate could not have resulted from a non-specific effect on

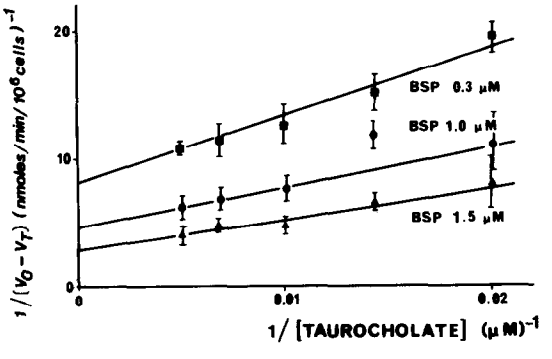

Fig. 3. Determination, according to Inui and Christensen of BSP uptake inhibited by Na^+-taurocholate. The initial uptake velocities were determined in the absence (V_0) and in the presence of different taurocholate concentrations (V_T). The intercepts on the y-axis represent the reciprocal values of BSP initial uptake sensitive to taurocholate at different BSP concentrations. The data of Fig. 2 were used for this representation.

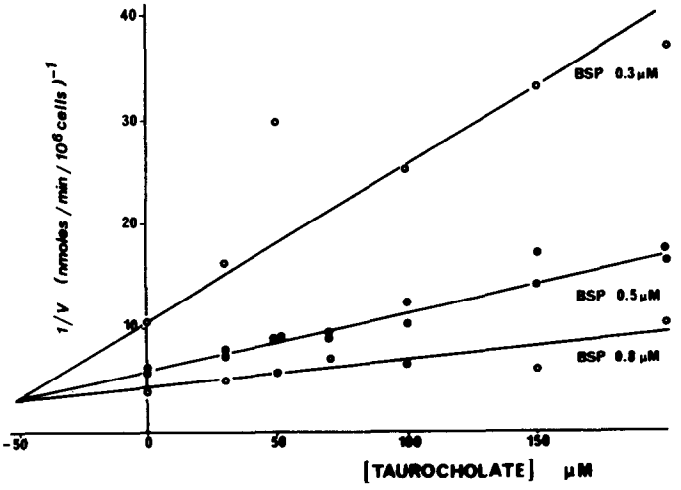

Fig. 4. Dixon plot of the inhibition by taurocholate of taurocholate sensitive component of BSP uptake. Taurocholate sensitive BSP uptake was obtained as indicated in Fig. 3 for each BSP concentration. This value minus the decrease in the BSP uptake rate (V_0–V_T) obtained at each taurocholate concentration, gives the remaining taurocholate sensitive BSP uptake which is plotted against taurocholate concentration.

the cellular membrane, such as a detergent effect, since taurocholate does not change the intracellular K^+ content, as previously shown [8]. In addition 2 mM Na^+-taurocholate (ten times the higher concentration used in this study) is without effect on lactate dehydrogenase release from lymphocyte cells after a ten minute exposure at 37°C [9]. The competition observed between Na^+-taurocholate and BSP, and the absence of effect of taurocholate on other carriers of the cell membrane [10] are in accordance with a specific effect of taurocholate at the sites involved in BSP uptake. Several authors, however, have not observed such an effect of taurocholate or other bile salts, on BSP or bilirubin uptake [1–3]. This discrepancy can be explained by the choice of concentrations used; for instance, in isolated rat liver cells it would be difficult to detect any effect of taurocholate using BSP concentrations higher than 10 μM, since at these concentrations the taurocholate sensitive component of BSP uptake represents less than 20% of the total uptake. Recently it has been reported that taurocholate can decrease the amount of BSP taken up by the rat liver [11] and of dibromosulfophthalein (DBSP) taken up by isolated rat liver cells [8].

By means of taurocholate inhibition, it is possible to divide the BSP uptake of rat liver cells into two components. Substraction of the high affinity component from total uptake showed that both components are saturable in relation to BSP concentrations (data not shown). This heterogeneity in the uptake of BSP can not be revealed by usual kinetic plots, even when a wide range of BSP concentrations is used, probably because of the small difference

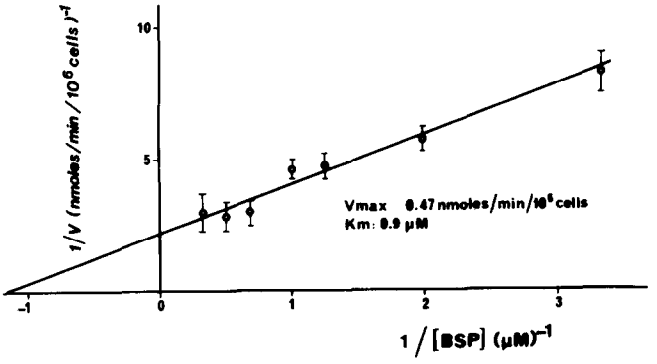

Fig. 5. Lineweaver Burk plot of the taurocholate sensitive component of BSP uptake. For each BSP concentration, the values of the BSP initial uptake sensitive to taurocholate were obtained from the intercepts on y-axis of Inui and Christensen plot as indicated in Fig. 3. Each value is indicated with the SEM calculated from three to five experiments. K_m and V_{max} values of 0·9 μM and 0.47 nmole/min/10^6 cells were obtained.

between the affinity constants of the two systems. The taurocholate sensitive component of BSP uptake is likely to be carried out by the Na^+-independent carrier of bile salts, which can be competitively inhibited by BSP or bilirubin [4]. Such an hypothesis is supported by the close similarity which exists between the K_i value (50 μM) for taurocholate inhibition of BSP uptake obtained in the present study, and the K_m (57 $\pm$ 3.8 μM) of the Na^+-insensitive taurocholate uptake by rat liver cells [4].

The existence of several carriers for organic anions transport has already been postulated at the canalicular level of the hepatocyte [5, 12]. It is generally assumed that the pathways for excretion in bile of organic anions like BSP and bilirubin are not shared by bile salts [13, 14]. It has been shown, however, that glycodihydrofusidate, an analogue of bile salts, can inhibit both BSP and bile salt excretion in the bile [15] and the bile acid ester sulfates can inhibit DBSP excretion [16].

It remains hazardous to speculate on possible physiological consequences of a partially shared transport of organic anions and bile salts, primarily because both are bound to albumin. One may, however, assume that it plays some role in situation such as cholestasis, where there is a delayed plasma clearance of various anionic drugs. In fact it has been observed that infusion of a high dose of dehydrocholate in the dog can decrease the transfer rate of BSP from the plasma to the liver [17]. In man [18], kinetic data strongly suggest a transport of BSP very similar to that observed in the rat, and cholestasis again yields a diminution of the plasma- to- liver transport of the dye.

REFERENCES

1. B. F. Scharschmidt, J. G. Waggoner and P. D. Berk, *J. Clin. Invest.* **56**, 1280–1292 (1975).
2. G. Paumgartner and J. Reichen, *Clin. Sci.* **51**, 169–176 (1976).
3. M. Schwenk, R. Burr, L. Schwarz and E. Pfaff, *Eur. J. Biochem.* **64**, 189–197 (1976).
4. M. S. Anwer and D. Hegner, *Hoppe-Seyler's Z. Physiol. Chem.* **359**, 1027–1030 (1978).
5. R. Clarenburg and C. C. Kao, *Am. J. Physiol.* **225**, 192–200 (1973).
6. Y. Laperche, C. Graillot, J. Arondel and P. Berthelot, *Biochem. Pharmacol.* **28**, 2065–2069 (1979).
7. Y. Inui and H. N. Christensen, *J. Gen. Physiol.* **50**, 203–244 (1966).
8. R. J. Vonk, P. A. Jekel, D. K. F. Meijer and M. J. Hardonk, *Biochem. Pharmacol.* **27**, 397–405 (1978).
9. G. Holdsworth and R. Coleman, *Biochem. J.* **158**, 493–495 (1976).
10. J. K. Randolph and I. D. Goldman, *Proc. Am. Ass. Cancer Res.* **21**, 21 (abstract) (1980).
11. Z. Gregus and E. Fischer, *Arch. Int. Pharmacodyn.* **240**, 180–192 (1979).
12. J. L. Mahu, P. Duvaldestin, D. Dhumeaux and P. Berthelot, *Am. J. Physiol.* **232**, E445–E450 (1977).
13. S. Alpert, M. Mosher, A. Shanske and I. M. Arias, *J. Gen. Physiol.* **53**, 238–247 (1969).
14. S. Gutstein, S. Alpert and I. M. Arias, *Isr. J. Med. Sci.* **4**, 36–40 (1968).
15. Y. Delage, M. Dumont and S. Erlinger, *Am. J. Physiol.* **231**, 1875–1878 (1976).
16. N. B. Javitt, in *The Liver. Quantitative Aspects of Structure and Function* (Ed. G. Paumgartner, and R. Preisig) pp. 355–359. Karger, Basel (1973).
17. Y. Delage, S. Erlinger, M. Duval and J. P. Benhamou, *Gut* **16**, 105–108 (1975).
18. S. H. Quarfordt, H. L. Hilderman, D. Valle and E. Waddell, *Gastroenterology* **60**, 246–255 (1971).