Biochemical Pharmacology, Vol. 37, No. 16, pp. 3119–3126, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

SEX DIFFERENCES IN SULFOBROMOPHTHALEIN-GLUTATHIONE TRANSPORT BY PERFUSED RAT LIVER

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(*Received* 10 *August* 1987; *accepted* 8 *January* 1988)

Abstract—Sex differences have been described in the hepatic transport of many organic anions. Proposed mechanisms include differences in the rate of metabolism, in the degree of binding to cytoplasmic proteins, and in the rate of membrane transport. To better define these factors, we used the perfused rat liver to study the hepatic transport of the glutathione conjugate of sulfobromophthalein (BSP-GSP), a model compound that does not require metabolism for excretion. Hepatic transport of BSP-GSH was saturable for both sexes. Clearance of BSP-GSH from 1% albumin solutions at steady-state was 35–52% greater in female livers than in male livers, and reflected a 47% larger apparent V_{max} with no change in the apparent K_m. Analysis of the rate of disappearance of BSP-GSH from recirculating perfusate and its appearance in bile using a simple two-compartment model indicated that the ratio of influx to efflux was greater in female livers. These findings are compatible with sex-related differences in the electrochemical driving forces for BSP-GSH uptake.

Sex differences in hepatic transport have been described for a wide variety of xenobiotic and endogenous compounds, including oleate [1, 2], bilirubin [3, 4], sulfobromophthalein (BSP‡), indocyanine green [6–8], digoxin [9], taurocholate [10], and tartrazine [11]. In most reported cases, transport was more rapid in females. However, male rats excrete chlorothiazide more rapidly than females [7], and male hepatocytes have been reported to take up taurocholate [10] and some estrogen glucuronides [12] more rapidly than female cells.

Little is known regarding the specific transport mechanisms responsible for these sex differences. Indeed, the rate-limiting steps in the transport of most organic anions remain poorly defined [13–15]. Sex differences could thus reflect differences in the rate of transport from plasma into the liver cell, diffusional transport within the cell, metabolism, biliary excretion, or some combination of these processes. Most prior studies have not specifically addressed which of these steps is rate-limiting.

Extensive binding of organic anions to proteins or lipid membranes further complicates the interpretation of kinetic data. In the presence of binding, the difference between the total organic anion concentration inside and outside of the cell is a poor measure of the chemical gradient driving uptake, making it more difficult to determine if membrane transport is equilibrative or concentrative.

To minimize these problems, we chose to study transport of the glutathione conjugate of sulfobromophthalein (BSP-GSH), an organic anion that undergoes minimal hepatic metabolism, is rapidly excreted into bile, and is less avidly bound by plasma and cytoplasmic binding proteins than other organic anions. To maximize the chance of determining the specific transport process underlying any observed sex differences, we measured steady-state hepatic removal rates under both saturating and nonsaturating conditions and also determined the rate constants of the unidirectional transport steps.

METHODS

Materials. [^{35}S]Sulfobromophthalein (BSP) was from Amersham (Arlington Heights, IL; 20 mCi/mmol) and was more than 95% pure when assayed by the method of Whelan and Plaa [16]. Nonradioactive BSP, reduced glutathione, and bovine serum albumin (fraction V, essentially fatty acid free) were obtained from Sigma (St. Louis, MO). Oxypherol FC-43 was from Alpha Therapeutics (Los Angeles, CA). All other chemicals were of the highest grade commercially available.

Radiolabeled sulfobromophthalein-glutathione conjugate (BSP-GSH) was purified from bile collected after intravenous administration of approximately 3 μmol [^{35}S]BSP to a 55-day-old male rat under light ether anesthesia. Following administration, bile was collected in 5-min intervals, and the most radioactive samples (typically between 5 and 15 min) were purified by a modification of the method of Whelan and coworkers [17]. Briefly, bile was applied to Whatman No. 3 paper (Whatman Ltd., England) and chromatographed using a mixture of glacial acetic acid, water and *n*-propanol (1:5:10). BSP-GSH was localized by exposure of

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‡ Abbreviations: BSP, sulphobromophthalein; BSP-GSH, sulphobromophthalein glutathione conjugate; and FABP, fatty acid binding protein.

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the dried chromatograms to ammonia vapor, and the appropriate portions were cut out and eluted with distilled water. After filtration, samples were lyophilized, resuspended in 0.5 to 1 ml of water, and stored at −20° until used. Purity was greater than 95% when assessed by thin-layer chromatography [16]. Unlabeled BSP-GSH was prepared by the method of Whelan *et al.* [17] and consisted of approximately 95% monoconjugate and 5% diconjugate.

Liver preparation. Livers were removed under ether anesthesia from 55- to 65-day-old fed male or female Sprague–Dawley rats (Bantin Kingman, San Leandro, CA) by a modification of the procedure of Hems *et al.* [18] as previously described [19]. After mobilization, the liver was placed in a 37° thermoregulated cabinet and perfused via the portal vein with Oxypherol FC-43, a nontoxic blood substitute consisting of a 20% fluorocarbon emulsion in a bicarbonate-buffered balanced salt solution. Perfusate flow was regulated at 2.5 to 3 ml · min^{-1} · (g liver)$^{-1}$ using a precision peristaltic pump (Multiperpex, LKB, Sweden). A 30-min equilibration period of recirculating fluorocarbon perfusion preceded all experiments.

Single-pass perfusion. In single-pass studies, the circuit was then opened and the liver was perfused for 4 min with 1% albumin in Krebs–Henseleit buffer (equilibrated with 95% O_2, 5% CO_2) to remove the fluorocarbon from the liver. The liver was then perfused single-pass at 3.5 to 4 ml · min^{-1} · (g liver)$^{-1}$ with a series of test solutions containing specified concentrations of unlabeled BSP-GSH (5.6 to 100 μM) in the same buffer for 2 min each. For each solution, effluent samples were collected at 30-sec intervals for the first 2 min, a time sufficient to establish steady-state extraction. BSP-GSH concentrations in effluent and perfusate samples in single-pass studies were assayed by absorption at 580 nm following alkalinization of samples with NaOH. The single-pass extraction was calculated from the equation:

$$E = \frac{C_{in} - C_{out}}{C_{in}} \quad (1)$$

where C_{in} and C_{out} are the BSP-GSH concentrations entering and exiting the liver respectively.

The removal velocity for each BSP-GSH concentration was calculated from the equation:

$$V = E \cdot Q \cdot C_{in} \quad (2)$$

where Q is the perfusate flow rate per gram of liver and C_{in} is the concentration of BSP-GSH in the perfusate.

Calculation of intrinsic clearance. The intrinsic clearance (Cl) of BSP-GSH by the liver is defined as the volume of buffer that would be cleared of BSP-GSH by the liver per unit time in the absence of flow limitation [20].

We will assume that the hepatocytes in the lobule are exposed to a gradient of BSP-GSH concentrations ranging from the inflowing perfusate concentration to the outflowing concentration since we have previously demonstrated such gradients in our perfusion system [21]. For nonsaturating conditions, the intrinsic clearance is thus given by [22]:

$$Cl = Q \cdot \ln\left[\frac{C_{in}}{C_{out}}\right] \quad (3)$$

This equation was derived by assuming that the removal process is governed by a single rate constant. In fact, the removal rate at steady-state is determined by multiple rate constants. These include the rate constants for influx across the plasma membrane (k_1), efflux (k_2) and metabolic sequestration (k_3) [14]. However, at steady-state these three rate constants can be replaced by a single composite rate constant k defined by $k = k_1 \cdot k_3/(k_2 + k_3)$ [14, appendix in Ref. 23]. Thus, Equation 3 is valid for steady-state conditions.

Recirculating perfusion. In separate experiments, BSP-GSH uptake was studied from 50 ml of recirculating perfusate containing 1% bovine serum albumin in a commercial fluorocarbon emulsion. Bile flow was maintained at constant levels by infusion of 40 μM taurocholate into the reservoir at a rate of 10–20 nmol · min^{-1} · (g liver)$^{-1}$. After 30 min of perfusion, a tracer bolus of [^{35}S]BSP-GSH (less than 0.04 μmol) dissolved in 100 μl water was added to the recirculating perfusate (50 ml), and samples of perfusate (100 μl) and bile (total volume) were collected at intervals of 1 or more minutes for 90 min. The initial concentration of BSP-GSH in the perfusate (about 0.6 μM) was sufficient to provide adequate radioactivity for measurement, but was well below the K_m for BSP-GSH removal (about 38 μM, see below).

Sample radioactivity was determined by scintillation counting (Aquasol, New England Nuclear) using appropriate quench correction. Formation of metabolites was assessed by thin-layer chromatography of perfusate and bile samples in two experiments and was found to be less than 5% of the total added radioactivity. Therefore, metabolites were not quantitated in most experiments.

Assessment of viability. Viability of each liver was monitored by bile flow, perfusion pressure, appearance and (in single-pass studies) by redetermination of the BSP-GSH uptake rate at the end of the experiment using the initial perfusate solution. Viability in our perfusion system has been demonstrated by monitoring oxygen consumption, morphology by light and electron microscopy [19], and by membrane potential [24].

Binding assays. Binding of BSP-GSH to albumin and to the fluorocarbon beads was assessed by equilibrium dialysis. Three milliliters of Krebs tricine buffer [19] containing 20% fluorocarbon beads and/or 1% bovine albumin was dialyzed at 23° against 6 ml of the same buffer for 60 hr. Sodium azide (0.1%) was added to all buffers. BSP-GSH outside the bag was considered to reflect the equilibrium free BSP-GSH concentration, while BSP-GSH inside the dialysis bag was considered to reflect the total (bound plus free) concentration. The unbound fraction is the ratio of these values. Binding of BSP-GSH to FABP was assessed by its ability to displace radiolabeled fatty acid as previously described [25].

Assay of cytoplasmic binding protein levels. Following perfusion, livers were homogenized for 60 sec

in 0.1 M potassium phosphate (pH 6.5) using a Waring blender. Homogenates (1:4, w/v) were centrifuged at 20,000 *g* for 20 min, and the resulting supernatant fraction was centrifuged further at 100,000 *g* for 60 min. Supernatant fractions were stored frozen at −70° until assayed.

Levels of fatty acid binding protein (FABP) were measured by radial immunodiffusion as previously described using authentic rat liver FABP as standard [25]. Levels of the glutathione transferases were assessed by activity toward 1-chloro-2,4-dinitrobenzene, a substrate common to all forms of this enzyme [26]. Protein was measured by the method of Lowry *et al.* [27], using bovine serum albumin as standard.

Data analysis. In single-pass experiments, extraction was measured at 30, 60, 90 and 120 sec for each BSP-GSH concentration. Because steady-state was reached within 60 sec (see Results), the extractions at 60, 90 and 120 sec were averaged and used to calculate the steady-state uptake velocity according to Equation 2.

The effect of BSP-GSH concentration on the steady-state uptake velocity was analyzed by nonlinear least-squares computer curve fitting using a model consisting of a single saturable transport process. More complex models that included a second saturable or unsaturable transport process were also tested. However, these did not increase significantly the quality of the fit and typically resulted in a large uncertainty in the value of one or more parameters. Data for individual experiments were analyzed separately, and results were averaged to give the parameter values presented in the tables.

This method assumes that all cells within the liver are exposed to the concentration of BSP-GSH in the perfusate. This assumption may lead to overestimation of the K_m for highly extracted compounds due to formation of lobular concentration gradients. However, such errors are expected to be small for compounds with low extractions such as BSP-GSH, since the magnitude of the gradients formed is likewise small. Computer simulations of saturable uptake for compounds with low and high extractions suggest that the error is less than 10% for compounds with tracer extractions of up to 40% [28]. Since this includes the highest extractions measured in the current study, this source of error was neglected in our analyses.

Data from each recirculating experiment were analyzed by a modified Gauss–Newton regression method using a simple two-compartment model (Fig. 1). This model is the simplest of those tested that could adequately account for the data. Bile and plasma curves were fit simultaneously. Statistical significance was assessed by the unpaired *t*-test.

RESULTS

Single-pass liver perfusion. For each BSP-GSH concentration tested, extraction declined slightly between 30 and 60 sec and thereafter stabilized, indicating that steady-state had been achieved. Female livers removed BSP-GSH from the perfusate more efficiently than male livers at steady-state. In six to seven perfusions for each sex, extraction of a nonsaturating concentration of BSP-GSH (5.6 μM) was 20% higher in female livers ($P < 0.025$, Table 1).

Removal of BSP-GSH was saturable for both sexes (Fig. 2). Although the apparent K_m was similar ($P > 0.5$), the apparent V_{max} was 48% larger in females ($P < 0.01$, Table 1). This sex difference was fully evident at the earliest time points studied (Table 2).

In each sex, similar values for the apparent intrinsic clearance of BSP-GSH were obtained whether calculated from the single-pass extraction of BSP-GSH under nonsaturating conditions or from the V_{max}/K_m ratio (Table 3). These results suggest that the same transport step is rate-limiting to BSP-GSH removal under both saturating and nonsaturating conditions.

Recirculating liver perfusion. Both male and female livers rapidly cleared [^{35}S]BSP-GSH into the bile following its addition to the perfusate reservoir

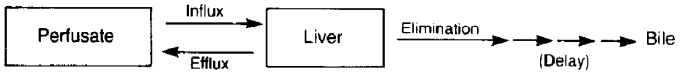

Fig. 1. Compartmental model used for analyzing the recirculating liver perfusion data.

Table 1. Extraction of BSP-GSH by single-pass perfused rat liver*

Sex	Extraction (%)	V_{max} (nmol·min^{-1}·g^{-1})	K_m (μM)	V_{max}/K_m (ml·min^{-1}·g^{-1})	Liver wt (g)	Flow (ml·min^{-1}·g^{-1})	N
Male	32.6 ± 2.1	46.5 ± 5.8	38.3 ± 3.2	1.17 ± 0.09	8.66 ± 0.74	3.76 ± 0.97	6
Female	39.2 ± 1.4	68.6 ± 5.6	37.0 ± 3.0	1.88 ± 0.15	7.42 ± 0.58	4.02 ± 1.20	7
	$P < 0.025$	$P < 0.01$	$P > 0.5$	$P < 0.005$	$P > 0.2$	$P > 0.35$	

* Isolated rat livers were perfused single-pass with Krebs–Henseleit buffer containing 1% albumin and graded concentrations of BSP-GSH. The listed extraction is the fraction of the BSP-GSH removed under nonsaturating conditions (5.6 μM). The uptake velocity for each concentration was calculated from the mean extraction between 1 and 2 min. The apparent K_m and V_{max} were determined from the concentration-velocity curve by nonlinear least-squares computer curve fitting. Mean values are shown with their standard errors.

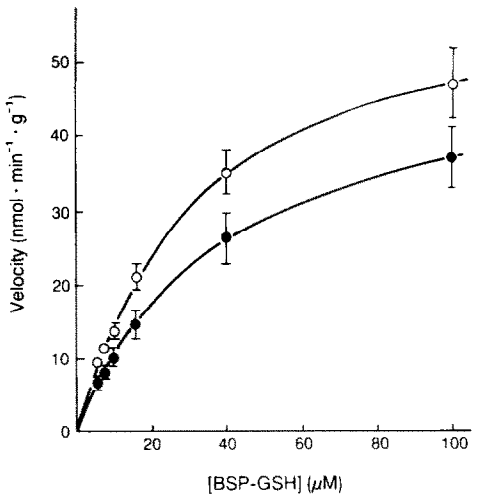

Fig. 2. Effect of BSP-GSH concentration on removal velocity. Male livers (●) and female livers (○) were perfused single-pass, and the initial extraction for each concentration was used to calculate the uptake velocity.

(Fig. 3). Thin-layer chromatography [16] demonstrated that over 95% of the biliary radioactivity was BSP-GSH, indicating little metabolism of the compound prior to excretion. A small amount of radioactivity (averaging less than 2% of the administered dose) remained in the liver at the end of the experiment. Because of the very small amount of radioactivity present, attempts to determine chromatographically whether this radioactivity represented metabolite or unchanged BSP-GSH were unsuccessful.

Data were analyzed using a compartmental model that includes the rate constants for influx, efflux, and biliary excretion (Fig. 1). In six to seven experiments for each sex, the apparent influx rate constant (per gram) averaged 32% greater in females and the apparent efflux rate constant averaged 27% smaller (Table 4). Although these differences are large enough to account for the observed sex differences in BSP-GSH uptake, neither was statistically significant alone ($P = 0.08$ and $P = 0.07$ respectively). However, their ratio, which determines the equilibrium partition of BSP-GSH between the cytosol and the plasma compartments, was significantly greater in females ($P < 0.01$). No sex difference was seen in the rate constant for elimination ($P > 0.05$).

The intrinsic clearance at steady-state is given by $k_1 \cdot k_3/(k_2 + k_3)$ [14, appendix in Ref. 23]. In order to express this rate constant as a clearance, it is necessary to multiply k_1 by the volume of the recirculating perfusate (50 ml) and to normalize for liver weight. The resulting values for intrinsic clearance agree well with the values determined from single-pass perfusion (Table 3).

Levels of cytoplasmic binding proteins. Fatty acid binding protein (FABP) levels were 54% greater in females ($P < 0.0001$, Table 5), while glutathione transferases (including ligandin) were 35% lower ($P < 0.025$), in agreement with previous reports [29, 30].

Table 2. Time dependence of removal in single-pass perfused liver*

Sex	Time (sec)	V_{max} (nmol·min^{-1}·g^{-1})	K_m (μM)	V_{max}/K_m (ml·min^{-1}·g^{-1})
Male	30	51.1 ± 9.0	43.9 ± 5.3	1.13
(N = 6)	60	46.1 ± 9.6	38.3 ± 4.8	1.16
	90	46.0 ± 10.5	37.1 ± 5.9	1.18
	120	47.4 ± 10.4	39.5 ± 5.8	1.18
Female	30	78.7 ± 8.3	44.7 ± 4.5	1.76
(N = 7)	60	70.0 ± 9.7	38.0 ± 5.4	1.87
	90	68.4 ± 9.3	36.9 ± 4.7	1.86
	120	67.3 ± 10.3	36.0 ± 5.7	1.90

* The apparent V_{max} and K_m values for BSP-GSH removal were determined using samples collected from 30 to 120 sec following exposure of the single-pass perfused liver to each BSP-GSH solution. Values are means ± SE.

Table 3. Comparison of intrinsic clearances at steady-state*

Perfusion system	Method	Intrinsic clearance (ml·min^{-1}·g^{-1}) Male	Female	Significance
Single-pass	$Q \cdot \ln(C_{in}/C_{out})$	1.48 ± 0.12	2.00 ± 0.09	$P < 0.025$
Single-pass	V_{max}/K_m	1.17 ± 0.09	1.88 ± 0.15	$P < 0.005$
Recirculating	$k_1 \cdot k_3/(k_2 + k_3)$	1.73 ± 0.22	2.54 ± 0.33	$P < 0.05$

* Intrinsic clearances were calculated by three different methods using two different experimental protocols (see text). Differences between methods were not statistically significant for either sex. Values for recirculating experiments were calculated from the rate constants for the individual steps given in Table 4 [to convert to comparable units, k_1 was multiplied by the perfusate volume (50 ml) and the final result was divided by the liver weight]. Values are means ± SE, N = 6–7.

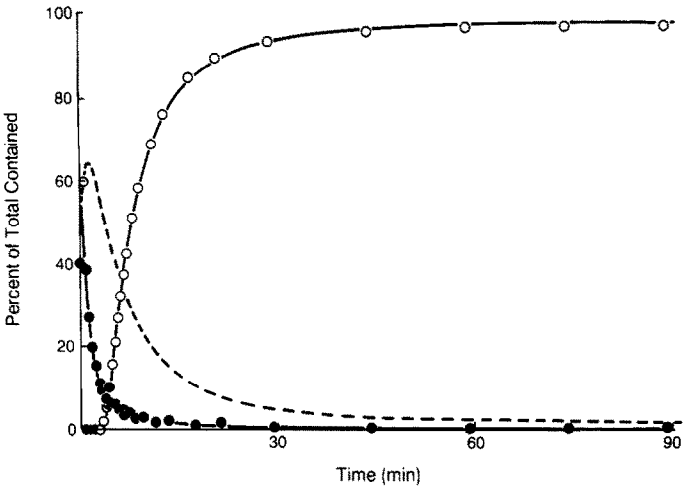

Fig. 3. Time course of BSP-GSH elimination by recirculating perfused rat liver. The plasma concentration (●) fell rapidly within the first few minutes after which the BSP-GSH appeared in the bile (○). The calculated concentration in the liver is also shown (broken line). The curves are the best fit of the model in Fig. 1 to representative data.

Binding studies. Equilibrium dialysis indicated that 92% of BSP-GSH was bound in the presence of 1% albumin, while no binding of BSP-GSH to Fluosol beads could be detected. Binding to FABP was less avid. Using a displacement assay [25], we found that concentrations of BSP-GSH as high as 1300 μM caused minimal displacement of oleate from FABP. In contrast, unconjugated BSP was much more effective (Fig. 4). The apparent K_d for binding of BSP-GSH to FABP determined from these inhibition curves by the method of Bass [25] was 12.7 ± 4.0 μM for BSP, and >1300 μM for BSP-GSH. Because this latter value exceeds the concentration of FABP in the liver [29], we conclude that little BSP-GSH is bound to FABP in hepatic cytosol.

Comparison of single-pass and recirculating data. Values for intrinsic clearance determined from the single-pass extraction of low concentrations of BSP-GSH were in excellent agreement with the value calculated from the rate constants determined in the recirculating liver (Table 3). This agreement further validates both methods.

It is also possible to calculate intrinsic clearance from the saturation data as the ratio of the apparent V_{max} to the K_m [22]. For both male and female livers, this ratio was in excellent agreement with the intrinsic

Table 4. Rate constants for uptake of BSP-GSH under nonsaturating conditions*

Sex	Influx (min^{-1})	Influx/g liver ($min^{-1} \cdot g^{-1}$)	Efflux (min^{-1})	Elimination (min^{-1})	Liver wt (g)	Flow ($ml \cdot min^{-1} \cdot g^{-1}$)	N
Male	0.443 ± 0.053	0.0506 ± 0.006	0.110 ± 0.015	0.240 ± 0.032	8.76 ± 0.35	2.63 ± 0.30	6
Female	0.470 ± 0.056	0.0670 ± 0.008	0.080 ± 0.009	0.250 ± 0.013	7.02 ± 0.25	2.89 ± 0.30	7
	P > 0.5	P = 0.08	P = 0.07	P > 0.5	P < 0.025	P > 0.5	

* Mean ± standard error values for the apparent rate constants are given for the best fit of the model shown in Fig. 1 to the recirculating liver perfusion data, and are given as the fraction of the relevant pool exchanged by the indicated process per minute. To compare influx rate constants for male and female livers, it was necessary to divide each by the liver weight to compensate for differences in liver size.

Table 5. Cytoplasmic levels of FABP and glutathione transferases*

	FABP		Glutathione transferases		
	($mg \cdot g^{-1}$)	($mg \cdot liver^{-1}$)	($nmol \cdot min^{-1} \cdot g^{-1}$)	($\mu mol \cdot min^{-1} \cdot liver^{-1}$)	N
Males	5.06 ± 0.19	45.7 ± 3.2	7370 ± 340	64.2 ± 2.3	6
Females	7.78 ± 0.30	54.6 ± 2.0	4810 ± 960	34.3 ± 5.3	7
	P < 0.0001	P < 0.025	P < 0.025	P < 0.001	

* Fatty acid binding protein (FABP) levels were assayed by immunodiffusion, whereas glutathione transferases (including Ligandin) were assayed by glutathione transferase activity toward 1-chloro-2,4-dinitrobenzene. Values are means ± SE.

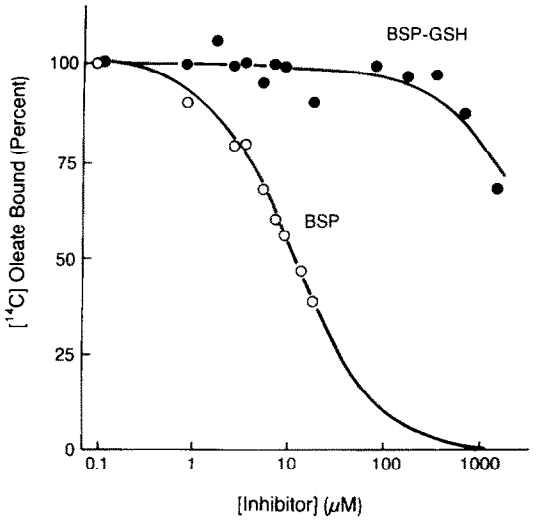

Fig. 4. Displacement of oleate from fatty acid binding protein by BSP and BSP-GSH.

clearance calculated by the other methods. This result indicates that the same removal step that is rate-limiting at low BSP-GSH concentrations also limits the uptake rate under saturating conditions.

DISCUSSION

Despite intensive investigation, the mechanism by which the liver extracts endogenous and exogenous amphipathic and lipophilic compounds from the plasma remains incompletely understood and the rate-determining processes largely undefined [13]. In part, this reflects the fact that transport of organic anions is a much more complicated process than transport of most water soluble compounds. While transport of hydrophilic compounds is almost always determined by movement across the hydrophobic core of the cell membrane, amphipathic and hydrophobic compounds such as organic anions and their conjugates must surmount several other barriers. These include diffusion across aqueous layers on either side of the cell membrane [31] and dissociation from binding sites on membranes and proteins [14]. For these reasons, it cannot be assumed that transport across the plasma membrane is rate-limiting to removal of organic anions. Although membrane carrier proteins have been described recently for BSP [32–34], bilirubin [32–34] and fatty acids [35] that are believed to facilitate transport across the plasma membrane, this does not necessarily indicate that plasma membrane transport is rate-limiting. Indeed, by catalyzing membrane transport, these carriers simply increase the probability that other less rapid steps in the transport sequence will determine the rate of hepatic removal.

Naturally occurring sex differences in hepatic transport offer an opportunity to gain new insights into transport events. However, interpretation of the available data is complicated by the large number of possible explanations that must be considered. At least six different processes collectively determine the rate of hepatic removal of organic anions from plasma at steady-state: plasma flow, dissociation from albumin, influx, efflux, cytoplasmic diffusion, metabolism, and (for cholephilic compounds) biliary excretion.

We chose to study BSP-GSH because its relative lack of metabolism and high water solubility greatly simplify data interpretation. These properties are also shared by dibromosulfophthalein (DBSP), which has been studied extensively for this reason [36–41]; however, the absence of a commercially available radiolabeled form of DBSP and the relative insensitivity of spectrophotometric assays precluded use of DBSP in the recirculating experiments. We compared results of two entirely different experimental protocols because data obtained under more restricted experimental conditions are often insufficient to determine which transport step is rate-limiting at steady-state [14]. Our approach allows certain conclusions to be drawn regarding the relative importance of each transport step in determining the removal rate and, therefore, the observed sex difference.

Flow. Difference in the rate of perfusate flow cannot account for the observed sex difference since the flow rate (per g) was the same for male and female livers. Moreover, a necessary condition for flow to be rate-limiting is a high first-pass extraction [20]. Since the sex difference was detected even under saturating conditions where the first-pass extraction did not exceed 15%, plasma flow cannot be responsible.

Dissociation. Although we have shown recently that dissociation from albumin can be rate-limiting to organic anion uptake under certain conditions [14], differences in the rate of dissociation within the liver appear unlikely to account for the sex difference in BSP-GSH uptake. BSP-GSH is a relatively water-soluble organic anion that is bound by albumin much less avidly than most other organic anions and should, therefore, dissociate rapidly.

Membrane transport. Because of its hydrophilic nature, BSP-GSH probably requires a membrane carrier to enter the cell. Indeed, the data provided by our study and a previous report [42] conform to carrier-mediated kinetics. The observed sex difference might be explained if female livers either contained a greater number of these carriers or if the carriers cycled more efficiently. This explanation is consistent with our finding of a greater apparent V_{max} for BSP-GSH transport in females. However, such a mechanism would be expected to affect influx and efflux equally and cannot, therefore, explain the increased ratio of the influx to efflux rate constants.

Our data are most consistent with a sex-related difference in the electrochemical driving forces for BSP-GSH uptake. Recent data suggest that transport of organic anions may be electrogenic [43] and may occur by anion exchange [44, 45]. If so, sex differences might be explained if female liver cells had a less negative interior than male cells or if transport were coupled to a more favorable gradient of the intracellular ion involved in anion exchange. This hypothesis is consistent with recent preliminary data suggesting that female perfused livers have a less

negative membrane potential than male livers [46]. This explanation is also attractive because such a mechanism would have opposite effects on influx and efflux, resulting in the increased ratio of influx/efflux detected in this study.

Steady-state removal rates could also be increased if efflux were reduced without affecting the influx rate. This might be accomplished by reducing the free concentration of the transported compound within the cell through an increase in binding to membranes or proteins [47]. However, such a mechanism can reduce only initial rates of efflux, since once the storage capacity of the binding sites is exhausted, the free concentration within the cytoplasm will rise to the same steady-state level it would have reached if no binding sites were present. The sex difference in the current study did not decline as steady-state was established (Table 2). Moreover, the only major binding protein whose level was greater in females was FABP, which displays such a low affinity for BSP-GSH that almost no cytoplasmic BSP-GSH would be bound to this protein. Increased cytoplasmic binding in females, therefore, seems unlikely to account for the observed differences.

Metabolism and excretion. Metabolism is not required for excretion of BSP-GSH by the liver and, therefore, cannot account for the observed sex difference. Biliary excretion is known to be rate-limiting to the hepatic removal of high concentrations of certain cholephilic molecules at steady-state, but seems unlikely to account for the current data. We have shown previously that, for elimination to be rate-limiting at steady-state, the rate constant governing elimination must be much smaller than the rate constant for efflux [14]. Since the opposite was true in the current study (Table 4), biliary excretion is unlikely to contribute to the sex differences seen in the current study. Moreover, no sex difference in the elimination rate constant was detected by compartmental modeling.

In summary, we have described a sex-related difference in the transport of conjugated sulfobromophthalein that is evident under both non-saturating and saturating conditions. The greater rate in females appears to be due to more efficient transport across the plasma membrane. The molecular mechanisms underlying this difference are not known. However, the data are most consistent with a more favorable electrochemical driving force for the movement of BSP-GSH into the cell in females, perhaps reflecting a less negative plasma membrane electrical potential difference in females.

Acknowledgements—We thank Dr. N. M. Bass for providing the binding and immunodiffusion assays. This study was supported by individual research grant DK 32898 and Liver Core Center Grant DK 26743 from the National Institutes of Health and by grants from the Academic Senate of the University of California and the Assessorato Pubblica Istruzione, Regione Sardegna, Italy.

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