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Multiple Carriers for Uptake of [^{3}H]Estradiol-17β(β-D-glucuronide) in Isolated Rat Hepatocytes

KIM L. R. BROUWER,[1] SHERRIE DURHAM, and MARY VORE

Department of Pharmacology, University of Kentucky College of Medicine, Lexington, Kentucky 40536

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SUMMARY

The transport of the cholestatic steroid glucuronide, ^{3}H-estradiol-17β-(β-D-glucuronide) ($E_2$17G), was examined in isolated female rat hepatocytes over a broad substrate concentration range (0.1–100 μM). Two different carrier systems were identified with the following kinetic parameters: Km_1 = 4.54 μM; $Vmax_1$ = 0.149 nmol/min/mg protein; Km_2 = 149 μM; $Vmax_2$ = 0.641 nmol/min/mg protein. Taurocholate and testosterone glucuronide selectively and competitively inhibited [^{3}H]-$E_2$17G uptake at the high affinity site. K_i values calculated for taurocholate (43 μM) and testosterone glucuronide (28 μM) indicated that these two inhibitors were relatively weak competitors for this $E_2$17G transport site. Conversely, $E_2$17G inhibited [^{3}H]taurocholate uptake into isolated hepatocytes (K_i = 43 μM). Bromosulfophthalein (10 μM) inhibited uptake of 0.5–50 μM [^{3}H]-$E_2$17G by 55–85%, whereas morphine glucuronide (100 μM) had no significant effect on uptake of [^{3}H]-$E_2$17G at these concentrations. The effects of taurocholate, testosterone glucuronide, bromosulfophthalein, and morphine glucuronide on [^{3}H]-$E_2$17G uptake into isolated rat hepatocytes correlated with the ability of these agents to inhibit binding of [^{3}H]-$E_2$17G to specific sites in rat liver plasma membranes. These data support the postulate that the two [^{3}H]-$E_2$17G binding sites identified in female rat liver plasma membranes represent two distinct organic anion carriers and indicate that the high affinity site for [^{3}H]-$E_2$17G represents a carrier that is shared by organic anions and bile acids.

The D-ring glucuronide conjugate of estradiol, $E_2$17G, induces a rapid, dose-dependent, and reversible decrease in bile flow in female rats when administered *in vivo* (1). This steroid glucuronide is an organic anion that is transported across the sinusoidal membrane into the hepatocyte by a carrier-mediated process. Brock and Vore (2) have shown that the uptake of [^{3}H]-$E_2$17G into isolated rat hepatocytes is saturable, is at least partially dependent on metabolic energy and an intact sodium gradient, and is inhibited by other organic anions such as dibromosulfophthalein and BSP.

Recent data have shown two specific binding sites for [^{3}H]-$E_2$17G in rat liver plasma membranes (3). The good correlation between organic anion inhibition of [^{3}H]-$E_2$17G binding (3) and uptake (4), observed in rat liver plasma membranes and hepatocytes, respectively, suggested that the low affinity binding site represented a non-bile acid organic anion carrier. The functional meaning of the high affinity binding site identified in rat liver plasma membranes was unclear, however, since previous studies by Brock *et al.* (4) indicated that taurocholate (200 μM) did not inhibit the uptake of [^{3}H]-$E_2$17G (100 μM) into isolated rat hepatocytes.

The present studies were designed to examine the kinetics of [^{3}H]-$E_2$17G uptake into isolated rat hepatocytes over a 1000-fold concentration range to determine if more than one uptake site could be identified. The effect of taurocholate, testosterone glucuronide, BSP, and morphine glucuronide on [^{3}H]-$E_2$17G uptake was also examined and correlated with the ability of these agents to inhibit [^{3}H]-$E_2$17G binding in rat liver plasma membranes.

Materials and Methods

Chemicals. [^{3}H]-$E_2$17G (50 Ci/mmol, >98% radiochemically pure after high-performance liquid chromatographic separation) and [^{3}H]-taurocholate (6.8 Ci/mmol, >98% radiochemically pure) were purchased from New England Nuclear (Boston, MA). Unlabeled $E_2$17G, taurocholic acid, cholic acid, testosterone glucuronide, and morphine glucuronide were purchased from Sigma Chemical (St. Louis, MO). BSP was obtained from Hynson, Westcott, and Dunning (Baltimore, MD). Collagenase (class II, 203 units/mg) was obtained from Cooper Biomedical (Freehold, NJ), and silicone oil (1.05 gm/ml) was purchased from Aldrich Chemical (Milwaukee, WI). All other chemicals were of reagent grade.

Animals. Female Sprague-Dawley rats (210–265 g, Harlan Industries, Indianapolis, IN) were used as liver donors for preparation of

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[1] Present address: Division of Pharmaceutics, School of Pharmacy, Beard Hall #10, The University of North Carolina, Chapel Hill, NC 27514.

ABBREVIATIONS: $E_2$17G, estradiol-17β-(β-D-glucuronide; BSP, bromosulfophthalein

hepatocytes. Rats were housed in stainless steel wire cages with free access to food and water and maintained on a 12-hr light/dark cycle.

Preparation of isolated hepatocytes. Hepatocytes were prepared by perfusion with collagenase as described previously (2). The cells (10–30 mg of cellular protein/ml of incubation buffer) were stored briefly on ice (0–4 hr) in an incubation buffer consisting of modified Hank's buffer with 10 mM Tris-HCl (pH 7.4). Protein was determined by the method of Lowry *et al.* (5) using bovine serum albumin as the standard. Cellular viability was determined by trypan blue exclusion on every preparation before and after each experiment. Viability ranged from 82 to 98% with a mean viability of 90%.

Experimental design. [^{3}H]-$E_2$17G (240 nCi) and various amounts of unlabeled $E_2$17G were incubated alone or in the presence of taurocholate, testosterone glucuronide, BSP, or morphine glucuronide with isolated hepatocytes (0.7–1.3 mg of cellular protein/ml of incubation buffer) in a total volume of 4 ml. To determine the effect of $E_2$17G on taurocholate uptake, [^{3}H]-taurocholate and various amounts of unlabeled taurocholate were incubated alone or in the presence of $E_2$17G with isolated hepatocytes (0.7–1.3 mg of cellular protein/ml of incubation buffer) in a total volume of 4 ml. After 5 min preincubation, the radiolabeled substrate was added to the hepatocyte suspension in 0.1 ml of vehicle. Taurocholate was dissolved in incubation buffer, while the vehicle for $E_2$17G consisted of incubation buffer: propylene glycol:ethanol (10:4:1, by volume). Preliminary experiments indicated that this vehicle has no effect on cell viability or uptake. Ten sec before addition of the radiolabeled substrate, 0.1 ml of the compound being examined, dissolved in incubation buffer, was added to the incubation flask. At 15, 30, 45, and 75 sec, a 0.2-ml sample was taken from the incubation flask and added to a 0.4-ml polyethylene tube containing 0.05 ml of 3 M KOH under a layer of 0.1 ml of silicone oil (1.05 g/ml silicone oil was combined with 0.863 g/ml mineral oil to obtain a final density of 1.02 g/ml). The tube was immediately spun in a tabletop microfuge B (Beckman Instruments, Fullerton, CA) for <30 sec. The tube was then cut at the silicone oil layer, and the hepatocyte pellet was placed in a scintillation mini-vial. The radioactivity of the hepatocyte pellet was determined after addition of 5 ml Scintiverse II (Fisher Scientific, Fair Lawn, NJ). Adherent fluid volume and cellular water volume were determined by the method of Baur *et al.* (6) by incubating cells with [carboxyl-^{14}C]dextran or [^{3}H]H_2O, respectively. Additionally, radioactivity in the cell-free supernatant was determined by scintillation counting of 0.05 ml supernatant, taken before cutting at the silicone oil layer, with 0.05 ml 3 M KOH and 5 ml scintillation cocktail.

Data analysis. The initial rate of uptake of [^{3}H]-$E_2$17G and [^{3}H]-taurocholate was determined from the slope of the least-squares regression line from 15–75 sec. This method does not consider as uptake the specific and nonspecific binding to cell membranes that occurs at zero time, as has been described (2, 7). Previous studies indicated that the uptake of [^{3}H]-$E_2$17G and [^{3}H]-taurocholate is linear for approximately 90–150 sec in isolated hepatocytes from female Sprague-Dawley rats (2). A general-purpose nonlinear curve fitting computer program (NONLIN) (8) was used to fit the uptake velocity versus substrate concentration data and obtain estimates for the Michaelis-Menten constants, V_{max} and K_m. Two-way analysis of variance with linear regression was used to determine when uptake was significantly decreased by an inhibitor based on Dixon plot analysis (i.e. when the slope of the regression line was significantly different from zero). To calculate K_i values, data from all concentrations in the Dixon plot that exhibited a significant slope based on weighted least-squares regression were pooled and fitted to a model that forced lines to have a common intersection. The estimated point of common intersection (K_i) with standard error and 95% confidence intervals is reported for each Dixon plot.

Results

The uptake of [^{3}H]-$E_2$17G and [^{3}H]-taurocholate into isolated female hepatocytes from Sprague-Dawley rats was linear from 15 to 75 sec as shown in Fig. 1. Linear regression analysis of the individual data yielded a positive y intercept and slope equivalent to the initial rate of uptake of the compound. Correlation coefficients were consistently above 0.9.

The relationship between initial uptake velocity and extracellular $E_2$17G concentrations ranging from 0.1 to 100 μM followed Michaelis-Menten kinetics (Fig. 2). Michaelis-Menten parameters were estimated from the untransformed data in this nonlinear system using the least-squares regression program NONLIN (8) ($1/Y^2$ weighting factor). The standard Michaelis-Menten equations for one site $V = [V_{max} * S]/[K_m + S]$ and for two sites $V = \{[V_{max} * S]/[Km_1 + S]\} + \{[Vmax_2 * S]/[Km_2 + S]\}$ were used in fitting the data. Using this method, the equation for two sites described the relationship between the initial uptake velocity and $E_2$17G concentration significantly better than the equation for one site according to Akaike's Information Criterion (9). Values for K_m (μM) and V_{max} (nmol/min/mg protein) calculated using nonlinear regression analysis for the two site equation were (mean ± SD): $Km_1{}^2c_1 = 4.54 \pm 2.50$, $Vmax_1 = 0.149 \pm 0.095$; $K_{m_2} = 149.4 \pm 410.9$, $V_{max_2} = 0.641Km_2 \pm 1.00$. The SD here represents the variability in the

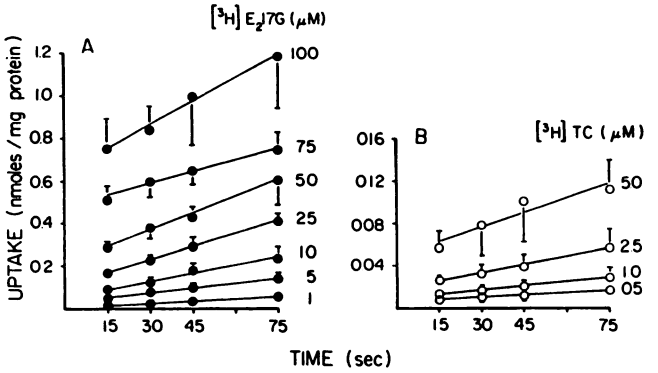

Fig. 1. Uptake of [^{3}H]-$E_2$17G (A) and [^{3}H]-taurocholate (TC) (B) into hepatocytes isolated from female rats. Timed samples were removed and uptake determined as described under Materials and Methods. Each point represents the mean ± SE of three to six experiments.

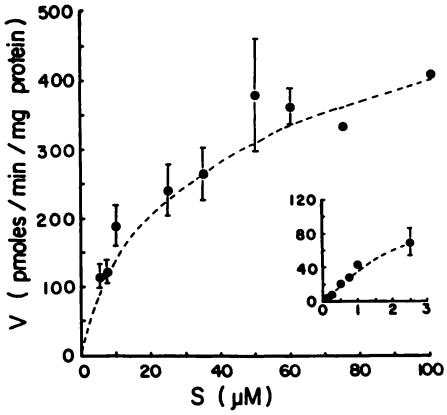

Fig. 2. Initial $E_2$17G uptake velocity (pmol/min/mg protein) by isolated rat hepatocytes versus extracellular $E_2$17G concentrations (0.1, 0.25, 0.5, 0.75, 1.0, 2.5, 5.0, 7.5, 10, 25, 35, 50, 60, 75, and 100 μM). The Michaelis-Menten equation for two sites was fitted to the data using NONLIN ($1/y^2$ weighting factor). Each point represents the mean ± SE of two to four experiments. The – – –, computer-generated best fit uptake curve when the two-site model was fit to all data points simultaneously. The *inset* shows initial uptake at low substrate concentrations.

estimates of the kinetic parameters when the two-site model was fit to all data points simultaneously. When the individual experiments (n = 3) were fit to the two-site model, the mean values (±SD) of the kinetic parameters were Km_1 = 4.26 ± 2.28, $Vmax_1$ = 0.148 ± 0.068; Km_2 = 422 ± 321, $Vmax_2$ = 1.29 ± 0.64. The SD in this case represents the between-preparation variability in the estimates. The high variability in the estimates of the high-capacity low affinity site (Km_2, $Vmax_2$) is due to the inability to test higher substrate concentrations of $E_2$17G because of its low solubility. The contribution of both the high and low affinity carriers to total [^{3}H]-$E_2$17G uptake at various $E_2$17G concentrations was calculated based on the Michaelis-Menten equation for two sites and the estimates for V_{max} and K_m. At $E_2$17G concentrations of 100, 40, 10, 5, 2.5, and 0.5 μM, the contribution of the high affinity site to total uptake is 36, 50, 72, 79, 83, and 88%, respectively.

The uptake of [^{3}H]-$E_2$17G into isolated hepatocytes was also examined in the presence of various inhibitors. Dixon plot analysis (Fig. 3) revealed that taurocholate (0, 0.5, 1.0, 2.5, 5.0, 10, 50, and 100 μM) inhibited [^{3}H]-$E_2$17G uptake primarily at low concentrations of $E_2$17G (0.5, 1.0, and 2.5 μM), where greater than 80% of total [^{3}H]-$E_2$17G uptake was due to the high affinity carrier. To calculate a K_i value, data from the Dixon plot were fitted to a straight line at each $E_2$17G substrate concentration using weighted least squares; a significant positive slope was observed at all $E_2$17G concentrations studied (0.5–100 μM). The common point of intersection (mean ± SE and 95% confidence intervals) using all concentrations is listed in Table 1. The mean K_i value for taurocholate inhibition of [^{3}H]-$E_2$17G uptake was 43 μM, and the lines intersected significantly above zero on the x axis (p = 0.0001), indicating that taurocholate competitively inhibited [^{3}H]-$E_2$17G uptake.

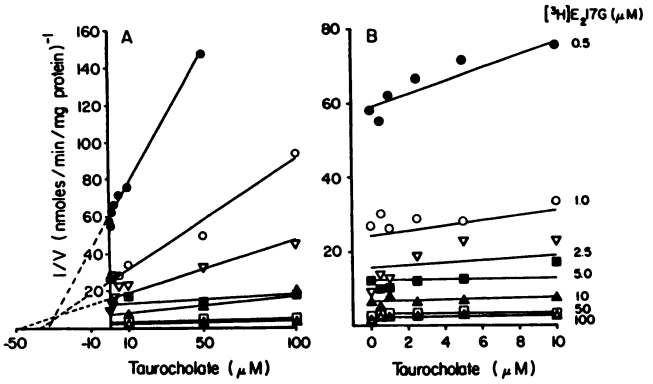

Fig. 3. A: Dixon plot of the effect of taurocholate (0, 0.5, 1.0, 2.5, 5.0, 10, 50, and 100 μM) on $E_2$17G (0.5, 1.0, 2.5, 5.0, 10, 50, and 100 μM) uptake into isolated rat hepatocytes. B: *Inset* of A showing $E_2$17G uptake at taurocholate concentrations ranging from 0 to 10 μM. Each point represents the mean of two to four experiments.

TABLE 1

K_i values for taurocholate and testosterone glucuronide inhibition of [^{3}H]-$E_2$17G uptake

Values were calculated from the respective Dixon plots (see Figs. 3 and 4).

Coordinates	Points of intersection	
	Taurocholate	Testosterone glucuronide
x		
Mean ± SE	−42.97 ± 4.62	−28.19 ± 4.75
95% CI[a]	−52.3 to −33.7	−37.6 to −18.8
y		
Mean ± SE	1.80 ± 0.45	2.78 ± 0.20
95% CI	0.904 to 2.70	2.39 to 3.16

[a] CI, confidence interval.

Testosterone glucuronide inhibition of [^{3}H]-$E_2$17G uptake into isolated hepatocytes was also examined. Dixon plot analysis (Fig. 4) indicated that testosterone glucuronide (0, 1.0, 5.0, 50, and 100 μM) inhibited [^{3}H]-$E_2$17G uptake at the lower concentrations of $E_2$17G (0.5, 2.5, 5.0, 10, and 50 μM) but not at 100 μM $E_2$17G. The weighted least-squares regression analysis also showed a significant slope at all concentrations except 100 μM $E_2$17G. Data from all concentrations except 100 μM were used to calculate a K_i value for testosterone glucuronide. The common point of intersection is listed in Table 1. The mean K_i value for testosterone glucuronide inhibition of [^{3}H]-$E_2$17G uptake was 28 μM, and the lines intersected significantly above zero on the x axis (p = 0.0001). As can be seen in Table 1, the confidence intervals of the K_i values for taurocholate and testosterone glucuronide overlap.

The data from the testosterone glucuronide inhibition study were plotted to show the uptake of [^{3}H]-$E_2$17G at the various concentrations in the absence and presence of 50 μM testosterone glucuronide (Fig. 5). The solid lines represent the theoretical uptake for the equation $V = [Vmax_1 * S]/[S + Km_1 (1 + I/K_I)] + [Vmax_2 * S]/[S + Km_2]$, where $Vmax_1$ and $Vmax_2$ are 0.149 and 0.641 nmol/min/mg protein, Km_1 and Km_2 are 4.5

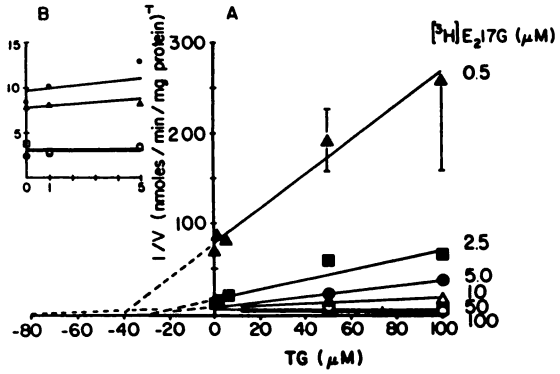

Fig. 4. A: Dixon plot of the effect of testosterone glucuronide (TG) (0, 1.0, 5.0, 50, and 100 μM) on $E_2$17G (0.5, 2.5, 5.0, 10, 50, and 100 μM) uptake into isolated rat hepatocytes. B: Inset of A showing $E_2$17G uptake at testosterone glucuronide concentrations ranging from 0 to 5 μM. Each point represents mean ± SE of three to five experiments.

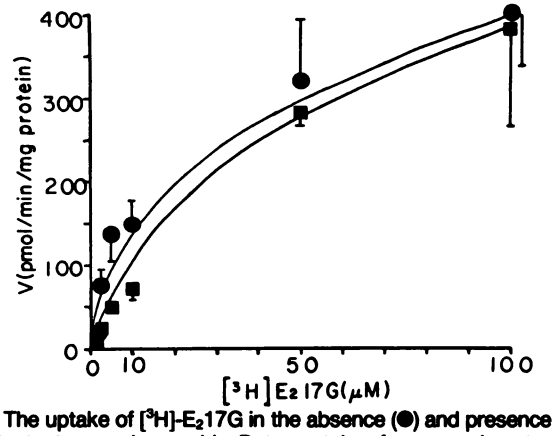

Fig. 5. The uptake of [^{3}H]-$E_2$17G in the absence (●) and presence (■) of 50 μM testosterone glucuronide. Data are taken from experiments shown in Fig. 4. *Solid lines* represent the theoretical uptake in absence (*top line*) and presence (*lower line*) of inhibitor using the equation for two sites with selective inhibition of the high affinity site as discussed in the text.

and 149 μM, and the K_I value for testosterone glucuronide is 28 μM. The good agreement between the observed and expected values for uptake in the presence and absence of inhibitor provides further evidence for the presence of two uptake sites, substantiates the selectivity of testosterone glucuronide for the high affinity site, and confirms that the calculated kinetic parameters are reasonable estimates.

Further studies were performed to determine if $E_2$17G also competitively inhibited taurocholate uptake. Dixon plot analysis (data not shown) indicated that $E_2$17G (0, 0.5, 5.0, and 50 μM) inhibited [^{3}H]taurocholate uptake into isolated hepatocytes at low concentrations of taurocholate (0.5, 1.0, 2.5, and 5.0 μM). The estimated point of common intersection using weighted least-squares regression was (x, y) (mean ± SE) −42.6 ± 10.67, 10.8 ± 7.95. The 95% confidence interval for the y intercept (−6.96 to 28.47) included the x axis, however, so that it cannot be stated with certainty that the inhibition is competitive.

Alterations in the uptake of [^{3}H]-$E_2$17G induced by BSP and morphine glucuronide were also studied in isolated hepatocytes (Table 2). Morphine glucuronide (100 μM) had no effect on [^{3}H]-$E_2$17G uptake, whereas BSP (10 μM) significantly inhibited [^{3}H]-$E_2$17G uptake into hepatocytes at all three concentrations of $E_2$17G studied.

Discussion

Previous studies in this laboratory have demonstrated that steroid D-ring glucuronide conjugates, such as $E_2$17G, are transported across the sinusoidal membrane into the hepatocyte by a saturable process. Brock and Vore (2) reported the following kinetic parameters for [^{3}H]-$E_2$17G uptake in isolated female rat hepatocytes: K_m = 145 ± 14.2 μM, V_{max} = 1.45 ± 0.20 nmol/min/mg protein ($E_2$17G concentrations ranged from 5 to 200 μM). Steroid glucuronides, such as estriol-16α-(β-D-glucuronide) and estradiol-17β-3-(β-D-glucuronide), and organic anions (i.e., dibromosulfophthalein and BSP) significantly inhibited [^{3}H]-$E_2$17G uptake into isolated hepatocytes, implying that $E_2$17G transport into the hepatocyte occurs by a carrier system shared by other steroid glucuronides and organic anions (4). However, taurocholate (200 μM) had no effect on the uptake of [^{3}H]-$E_2$17G (100 μM), indicating that a separate carrier is involved in taurocholate uptake in this concentration range (4).

Our laboratory has recently identified two specific binding sites for [^{3}H]-$E_2$17G in rat liver plasma membranes (3). To determine if the two binding sites identified in rat liver plasma membranes represented functional organic anion carriers, the kinetics of [^{3}H]-$E_2$17G uptake into isolated rat hepatocytes were reexamined over a wide range of substrate concentrations (0.1–100 μM). Substrate concentrations above 100 μM were not examined due to solubility limitations. The positive y intercept observed for both [^{3}H]-$E_2$17G and [^{3}H]-taurocholate uptake into isolated female rat hepatocytes (Fig. 1) may be attributed to specific and nonspecific binding to cell membranes (7). Preliminary experiments indicated that binding, but not uptake, of [^{3}H]-$E_2$17G in isolated rat hepatocytes occurs at 4°. Therefore passive diffusion of [^{3}H]-$E_2$17G into the hepatocyte was excluded as a non–carrier-mediated factor involved in uptake of [^{3}H]-$E_2$17G. These data are in agreement with the results of Brock and Vore (2) who showed that [^{3}H]-$E_2$17G uptake at higher concentrations could be described by Michaelis-Menten kinetics and showed no evidence of a diffusional component. This is in contrast to taurocholate, where significant diffusion into the hepatocyte occurs and uptake must be corrected for this non–carrier-mediated mechanism.

Michaelis-Menten constants were determined from nonlinear regression analysis of the data in Fig. 2, which was best described by the equation for two sites. These data unequivocally demonstrate that at least two components are involved in the carrier-mediated uptake of [^{3}H]-$E_2$17G into isolated rat hepatocytes.

The hypothesis that multiple carriers are involved in the hepatic uptake of organic anions is not new. At least two carriers have been identified for BSP (10) and taurocholate (11) uptake into hepatocytes. Photolabile bile acid derivatives have recently been synthesized and used to identify at least three domain-specific membrane proteins, two in the basolateral domain and a third in the canalicular membrane, involved in the uptake and excretion of bile acids and other organic anions (12, 13). The present transport data, in combination with the binding data (3), provide the strongest evidence for two organic anion carriers, one of which is shared with bile acids.

The effect of several bile acids and organic anions on [^{3}H]-$E_2$17G uptake was examined to characterize the substrate specificity of the two components of $E_2$17G uptake that had been identified. Taurocholate and testosterone glucuronide were chosen because of their selective inhibition of [^{3}H]-$E_2$17G binding to the high affinity site in rat liver plasma membranes (3). Based on Dixon plot analysis, both taurocholate and testosterone glucuronide are competitive inhibitors of [^{3}H]-$E_2$17G uptake with the lines intersecting above the x axis. The K_i values for taurocholate and testosterone glucuronide inhibition of [^{3}H]-$E_2$17G uptake were 43 and 28 μM, approximately 10- and 6-fold higher, respectively, than the Km_1, indicating that these agents are relatively weak competitors. Taurocholate and testosterone glucuronide were also selective inhibitors of the high affinity uptake site for $E_2$17G.

If taurocholate and $E_2$17G share a common carrier, then $E_2$17G would be expected to inhibit [^{3}H]-taurocholate uptake competitively. However, Brock *et al.* (4) showed that $E_2$17G had no effect on [^{3}H]-taurocholate uptake at higher concentrations of taurocholate (5, 20, 50, and 200 μM). Since more than one carrier is known to be involved in [^{3}H]-taurocholate uptake, we reexamined the effect of $E_2$17G on [^{3}H]-taurocholate uptake using low taurocholate concentrations (0.5–5.0 μM). The present data indicate that an $E_2$17G-sensitive component is involved in [^{3}H]-taurocholate uptake and strongly suggest that

TABLE 2
Inhibition of [^{3}H]-$E_2$17G uptake

	$E_2$17G concentration		
	0.5 μM	10 μM	50 μM
	nmol/min/mg protein		
Control	0.0205 ± 0.0033[a]	0.190 ± 0.021	0.302 ± 0.026
Bromosulfophthalein (10 μM)	0.003 ± 0.0005[b]	0.070 ± 0.015[b]	0.135 ± 0.019[b]
Morphine glucuronide (100 μM)	0.0208 ± 0.0016	0.183 ± 0.017	0.295 ± 0.032

[a] Values are means ± standard errors of three to six experiments.
[b] $p < 0.05$, different from control.

$E_2$17G competitively inhibited [^{3}H]-taurocholate uptake into isolated hepatocytes at these lower concentrations. These data support the postulate that taurocholate and $E_2$17G share a common carrier.

BSP (10 μM) significantly and profoundly inhibited [^{3}H]-$E_2$17G uptake into hepatocytes at all concentrations studied. BSP was also a potent inhibitor of [^{3}H]-$E_2$17G binding at both sites in rat liver plasma membranes (3). These data suggest that bromosulfophthalein and $E_2$17G share both carriers. Laperche *et al.* (10) examined the uptake of BSP into isolated rat hepatocytes and identified taurocholate-sensitive and taurocholate-insensitive components; the K_i for taurocholate inhibition of BSP uptake was 50 μM. The close similarity in K_i values for taurocholate inhibition of BSP uptake (50 μM) and $E_2$17G uptake (43 μM) and the presence of a taurocholate-insensitive carrier for both BSP and [^{3}H]-$E_2$17G uptake support the hypothesis that mutual carriers are involved in BSP and [^{3}H]-$E_2$17G uptake. Anwer and Hegner (11) identified two components in the uptake of [^{3}H]-taurocholate into isolated rat hepatocytes. The Na^+-independent low capacity component was found to be competitively inhibited by BSP. Laperche *et al.* (10) postulated that the taurocholate-sensitive carrier for BSP uptake identified in their studies may be the same as the Na^+-independent carrier for taurocholate. The present data support the hypothesis that this carrier is also the high affinity carrier for $E_2$17G.

Morphine glucuronide (100 μM) had no effect on [^{3}H]-$E_2$17G uptake in hepatocytes at all concentrations of $E_2$17G studied. These results are consistent with [^{3}H]-$E_2$17G binding data in rat liver plasma membranes, indicating that morphine glucuronide did not inhibit [^{3}H]-$E_2$17G binding at either site (3). Furthermore, these results are in agreement with data showing that morphine glucuronide is taken up very poorly into rat hepatocytes (14) or the isolated perfused rat liver (15).

Although the present data show that $E_2$17G can inhibit [^{3}H]-taurocholate uptake, it is unlikely that the cholestasis induced by $E_2$17G is due to inhibition of bile acid transport at this high affinity carrier. Increased concentrations of taurocholate would be expected to override the cholestatic effects of $E_2$17G since interaction at this site is probably competitive in nature, and the Na^+-dependent, high capacity carrier for taurocholate transport is not shared by $E_2$17G. This is consistent with recent studies by Adinolfi *et al.* (16) and Durham and Vore (17), indicating that higher concentrations of taurocholate protect against $E_2$17G-induced cholestasis. Furthermore, BSP, which also appears to share this high affinity carrier, has not been associated with cholestasis.

In summary, these results support the postulate that two carriers are involved in the uptake of [^{3}H]-$E_2$17G into isolated hepatocytes and that the two [^{3}H]-$E_2$17G binding sites identified in female rat liver plasma membranes represent these two organic anion carriers. The high affinity site for $E_2$17G represents a carrier that is shared by bile acids and organic anions, whereas the low affinity site represents an organic anion carrier that is not shared with bile acids.

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Send reprint requests to: Dr. Mary Vore, Department of Pharmacology, University of Kentucky College of Medicine, 800 Rose Street, Lexington, KY 40536