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EFFECT OF CHOLEPHILIC DYES ON HEPATIC TIGHT JUNCTIONAL PERMEABILITY IN THE RAT*

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Abstract—Changes in biliary permeability during cholephilic dye-induced choleresis, as assessed by measuring the movement into bile of two permeability probes, [^{14}C]sucrose and horseradish peroxidase, were analyzed following an i.v. infusion (60 nmol/min per 100 g body wt) of the model cholephilic organic anion sulfobromophthalein in rats. Dye infusion led to a progressive increase of the [^{14}C]sucrose bile-to-plasma ratio, which reached a maximum value after 100 min of dye infusion (+97%). Paracellular entry of horseradish peroxidase, as evaluated by the early peak of its biliary appearance curve, was also selectively increased (+69%), without changes in the later (transcytotic) access of the protein. Additional dose–response studies of biliary permeability to [^{14}C]sucrose, using sulfobromophthalein and rose bengal, showed that this effect was dose-dependent and rapidly reversed by interruption of dye administration. The influence of hydrophobic/hydrophilic balance on this effect was also studied by infusing four dyes covering a broad range of hydrophobicity (phenol red, bromocresol green, sulfobromophthalein, and rose bengal), so as to attain a similar value of dye hepatic content at the end of the experiment (≈150 nmol/g liver wt). Under these conditions, a strong positive correlation was found between the increase in biliary permeability to [^{14}C]sucrose and dye hydrophobicity. These results suggest that cholephilic dyes increase tight junctional permeability in a reversible and dose-dependent manner, and that this effect depends on the hydrophobic/hydrophilic balance of the dye.

Key words: tight junctions; biliary permeability; cholephilic dyes; sulfobromophthalein, horseradish preoxidase

Biliary permeability is closely related to the functional regulation and integrity of the tight junctions (zonulae occludens). These structures seal the lumen of bile canaliculi between adjacent hepatocytes, thus delineating the canalicular lumen and selectively retaining the bile content within it [1]. Evidence from physiological and morphological studies indicate that tight junctions have selective permeability for fluid and certain solutes, thus suggesting a regulatory role in bile formation [2]. In line with this view, permeability along this pathway was shown to be modulated by a variety of physiologic regulators of bile secretion such as bile acids [3–5] and various hormones [6]. However, the effect of changes in biliary permeability on bile flow does not appear to be simple. Whereas moderate increases in biliary permeability were suggested to be involved in choleresis induced by osmotic choleretics [3, 4, 7, 8], higher increases, enough to allow regurgitation of actively transported solutes from the canaliculus into the plasma, were proposed to be a primary cause of cholestasis [9]. This dual effect may help to explain why bile salts have both choleretic and cholestatic effects, depending on the dose administered [10].

Cholephilic dyes comprise a group of structurally related compounds that are selectively and efficiently transported by the liver. This property, as well as its easy quantitation by direct spectrophotometry, justifies their wide use as indicators of hepatic function. Additionally, they serve as an ideal experimental model of choleresis and cholestasis induced by drugs. In fact, like bile acids, cholephilic dyes induce either choleresis or cholestasis depending on the doses administered [11, 12]. Thus, it is conceivable that these compounds can influence biliary permeability as well. We addressed this issue by performing studies on the effect of BSP,‡ a model cholephilic dye, on biliary permeability by measuring the access into bile of two permeability probes, [^{14}C]sucrose and HRP. In addition, the influence of dye hydrophobicity was analyzed by performing comparative studies of [^{14}C]sucrose biliary access using three additional cholephilic dyes covering a wide range of hydrophobicity. Our results suggest that cholephilic dyes increase biliary permeability in a reversible and dose-dependent manner, and that the magnitude of this effect depends on the hydrophobic/hydrophilic balance of the dye.

MATERIALS AND METHODS

Animals and surgery

Adult male Wistar rats weighing 320–360 g were used. Before the experiments, the animals were maintained on a standard diet and water *ad lib.*, housed in a

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‡ Abbreviations: BSP, sulfobromophthalein; HRP, horseradish peroxidase; PR, phenol red; BCG, bromocresol green; RB, rose bengal; (1 − σ), sieving coefficient; and *k*, diffusion permeability coefficient.

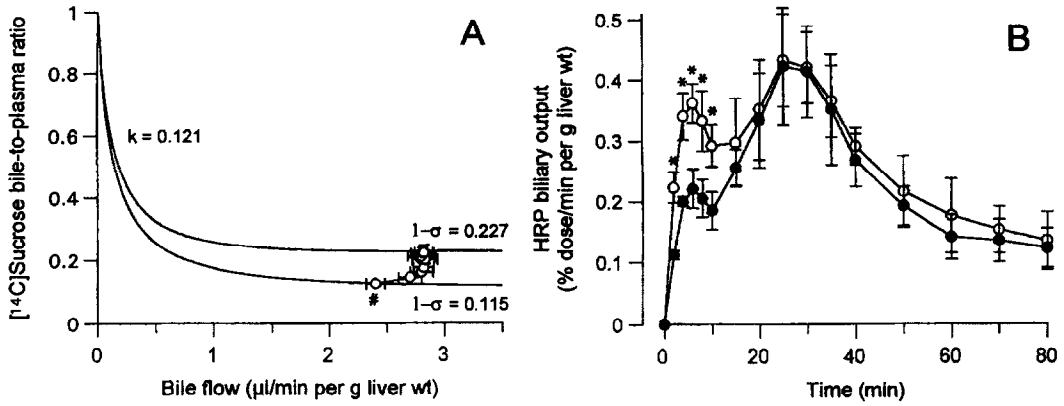

Fig. 1. (A) Relation between [^{14}C]sucrose bile-to-plasma ratio and bile flow during BSP administration. Following a basal collection period, BSP was infused (60 nmol/min per 100 g body wt), and blood and bile samples were collected in seven consecutive periods; the basal value is denoted with a number symbol (#). The bottom curve was obtained by fitting basal bile-to-plasma ratios and bile flow to Equation 3 (see Materials and Methods), assuming a sieving coefficient ($1 - \sigma$) of 0.115. The upper curve was calculated using the same equation, assuming a sieving coefficient of 0.227. The diffusion permeability coefficient (k) was set at 0.121. Values are means ± SEM of five experiments. (B) Biliary output of HRP during a BSP infusion of 60 nmol/min per 100 g body wt. HRP was administered at 100 min of dye infusion in five control (●) and five BSP-infused (○) rats. At this time, constant levels of bile flow and dye biliary output had already been reached. Values are means ± SEM of five experiments. Key: (*) significantly different from the control group ($P < 0.05$).

temperature- (21–23°) and humidity- (45–50%) controlled room under a constant 12-hr light–dark cycle.

The surgical procedure was performed so that bile collection was started between 9:00 and 11:00 a.m. to minimize the influence of circadian variations. Animals were anesthetized with a single dose of sodium pentobarbital (50 mg/kg body wt, i.p.), and were maintained in this condition throughout the experiment. The abdominal cavity was opened by a midline incision and, when [^{14}C]sucrose was administered, the renal pedicles were systematically ligated to prevent urinary excretion of the label. The common bile duct was then exposed and cannulated with a PE-10 polyethylene catheter (Intramedic, Clay Adams, Parsippany, NJ). Surgical preparation also included cannulation of the femoral vein (for fluid infusion) and the carotid artery (for blood withdrawal), using PC-50 polyethylene tubing (Intramedic). Body temperature was measured with a rectal probe connected to a constant temperature monitor, and maintained at 37.5–38.0° with an external heat lamp to prevent hypothermic alterations of bile flow. At the end of each experiment, the animals were killed by exsanguination, and then the livers were perfused with ice-cold saline, removed, and weighed.

Experimental procedure

Changes in biliary permeability were first evaluated by using [^{14}C]sucrose as a probe [3, 4, 13, 15]. For this purpose, 5 μCi of [^{14}C]sucrose was injected into the femoral vein after dissolving in 0.3 mL of saline, and flushed with 0.2 mL of solvent. A 60-min equilibration period was established to ensure that the distribution of the isotope between plasma and bile reached a steady state. Then, a basal period of 20 min was established followed by i.v. infusions of the dyes dissolved in 1% albumin in saline (or albumin–saline alone in controls). In dose–response studies, either BSP (30, 60, 90 and 120 nmol/min per 100 g body wt) or RB (4, 8, 13 and 18 nmol/min per 100 g body wt) was administered i.v. for 140 min. In reversibility studies, either BSP (90 nmol/

Table 1. Bile flow, [^{14}C]sucrose bile-to-plasma (BIP) ratio and calculated permeability parameters in BSP-infused rats

Time (min)	Bile flow (μL/min per g liver wt)	[^{14}C]Sucrose B/P ratio	$1 - \sigma$	k
Basal	2.44 ± 0.08	0.126 ± 0.003	0.115 ± 0.006	0.121 ± 0.008
0–20	2.69 ± 0.10*	0.145 ± 0.006*	0.139 ± 0.006*	0.208 ± 0.015*
20–40	2.80 ± 0.11*	0.161 ± 0.006*	0.157 ± 0.005*	0.282 ± 0.012*
40–50	2.82 ± 0.09*	0.175 ± 0.012*	0.172 ± 0.012*	0.200 ± 0.014*
60–80	2.83 ± 0.11*	0.201 ± 0.014*	0.200 ± 0.014*	0.422 ± 0.039*
80–100	2.80 ± 0.12*	0.220 ± 0.016*	0.219 ± 0.016*	0.472 ± 0.045*
100–120	2.81 ± 0.10*	0.225 ± 0.018*	0.224 ± 0.018*	0.513 ± 0.055*
120–140	2.82 ± 0.08*	0.228 ± 0.019*	0.227 ± 0.019*	0.535 ± 0.064*

After a basal bile collection period, BSP (60 nmol/min per 100 g body wt) was infused during the following 140 min. Sieving coefficients ($1 - \sigma$) were calculated by solving Equation 3 for $1 - \sigma$, assuming a diffusion permeability coefficient (k) of 0.121. The k values were calculated by solving Equation 3 for k, assuming $1 - \sigma$ to be 0.115. Initial values of $1 - \sigma$ and k were obtained by fitting basal [^{14}C]sucrose bile-to-plasma ratios and bile flow to Equation 3. Values are means ± SEM of five experiments.

* Significantly different from basal values ($P < 0.005$).

Table 2. Biliary cumulative output of HRP in control and BSP-infused rats

	Controls	BSP-Infused
Cumulative output (% dose per g liver wt) × 10^3		
First peak (0–10 min)	1.48 ± 0.23	2.51 ± 0.27*
Second peak (10–80 min)	18.39 ± 3.27	20.49 ± 3.66

After 100 min of a steady infusion of BSP (60 nmol/min per 100 g body wt), HRP (0.5 mg/100 g body wt) was injected into the portal vein. Bile was collected in appropriate sampling periods (2–10 min) for the remainder of the experiment. Quantitative resolution of para- and transcellular HRP access was achieved by calculating the cumulative output of the protein during the first peak (0–10 min) and the second peak (10–80 min) of the HRP biliary appearance curve, respectively. Values are means ± SEM of five experiments.

* Significantly different from control values ($P < 0.01$).

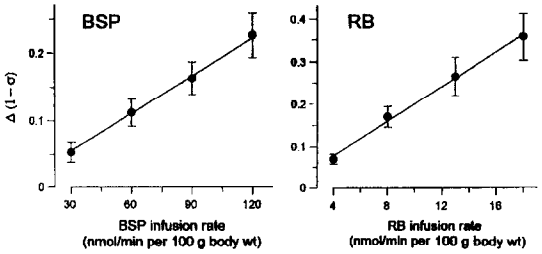

Fig. 2. Relationship between the changes in the sieving coefficient for [^{14}C]sucrose ($1 - \sigma$) and BSP infusion rates (30 nmol/min per 100 g body wt, N = 6; 60, N = 5; 90, N = 5; 120, N = 6) or RB infusion rates (4 nmol/min per 100 g body wt, N = 5; 8, N = 4; 13, N = 6; 18, N = 4). The respective basal sieving coefficients for BSP were 0.106, 0.115, 0.119 and 0.123, and the respective basal diffusion permeability coefficients were 0.126, 0.119, 0.124 and 0.122. For RB, basal sieving coefficients were 0.136, 0.124, 0.110 and 0.128, and basal diffusion permeability coefficients were 0.131, 0.125, 0.131 and 0.122. Final sieving coefficients were calculated by solving Equation 3 (see Materials and Methods) using the [^{14}C]sucrose bile-to-plasma ratio and the bile flow values obtained during the last (120–140 min) infusion period, and assuming that no change in the diffusion permeability coefficient occurred over the whole experimental period. Values are means ± SEM.

min per 100 g body wt) or RB (13 nmol/min per 100 g body wt) was administered i.v. during 100 min and then stopped; [^{14}C]sucrose bile-to-plasma ratios were further determined for the following 140 or 350 min for BSP and RB, respectively. Finally, to assess the influence of dye hydrophobicity on biliary permeability, four dyes were administered at different rates for 140 min so as to attain a similar dye hepatic content at the end of the experiment (≈150 nmol/g liver wt). The dyes administered were BSP, PR, BCG and RB, and the infusion rates were 90, 110, 30, and 13 nmol/min per 100 g body wt, respectively. During dye infusions, bile was collected at 20-min intervals, and blood was collected at the mean times of the bile-sampling intervals.

Quantitation of the early (paracellular) access of HRP into the bile was carried out to confirm by a separate method the effect of BSP administration on biliary permeability [15]. Details of this procedure, adapted to be used in the whole rat, have been described previously by our laboratory [16]. In brief, the animals received an i.v. steady infusion of BSP (60 nmol/min per 100 g body wt) for 160 min, and HRP (0.5 mg/100 g body wt) was injected into the portal vein 100 min after starting dye administration. Bile was collected in appropriate sampling periods (2–10 min) for the remainder of the experiment.

Analytical methods

Bile flow was determined gravimetrically assuming the specific gravity of bile to be 1.0. Dye biliary concentration was determined by direct spectrophotometry after an appropriate dilution of bile with water (BCG, RB) or 0.1 N sodium hydroxide (PR, BSP) at the following wavelengths: BCG: 600 nm, BSP: 580 nm, and RB: 550 nm. Total PR (non-conjugated plus PR-glucuronide) was determined spectrophotometrically at 560 nm after hydrolysis of the conjugated moiety of the dye according to the method of Hart and Schanker [17]. Biliary output was calculated by multiplying bile flow by biliary concentration after correcting for the transit time within the biliary tree and the bile duct catheter [18]. Dye liver content was assessed spectrophotometrically following extraction of the dye from the liver homogenate with 66% ethanol [19]. Recovery was consistently over 95%, irrespective of the dye considered.

The degree of hydrophobicity of the dyes was determined by reverse-phase HPLC, as reported previously [20]. A hydrophobicity index for dye X (HI_x) was calculated using the following equation [21]:

$$HI_X = \ln (R_X)/\ln (R_{RB}) \quad (1)$$

where R_X is the relative retention factor of the dye X, which is defined as follow:

$$R_X = (T_X - T_0)/(T_{PR} - T_0) \quad (2)$$

where T_X is the retention time for the dye X and T_0 is the retention time of the solvent front. Thus, an HI_X value of 0 is assigned to PR and a value of 1 is assigned to RB.

[^{14}C]Sucrose is plasma (100 μL) and bile (100 μL) was measured in a liquid scintillation counter (RackBeta, Pharmacia Wallac Oy, Finland), using OptiScint 'Hi Safe' (LKB, England) as a scintillation fluid. Quenching was corrected by the use of [^{14}C]toluene as an external standard. HRP in bile was determined spectrophotometrically by measuring the rate of oxidation of 4-aminoantipyrine at 510 nm [22].

Data analysis

The bile-to-plasma concentration ratio of [^{14}C]sucrose was determined for each collection period after correcting for the transit time within the biliary system and the bile duct tubing [18]. Basal sieving coefficients ($1 - \sigma$) and diffusion permeability coefficients (k) for each experimental group were determined by fitting the respective basal [^{14}C]sucrose bile-to-plasma ratios (B/P) and bile flows (F) to the following equation [4, 13, 14]:

$$B/P = (1 - \sigma)/[1 - \sigma \cdot \exp - ((1 - \sigma) \cdot F/k)] \quad (3)$$

Evaluation of the changes in biliary permeability during dye infusion was performed as previously described by others [4]. As Equation 3 states, changes in biliary permeability to the isotope could be due to an alteration of

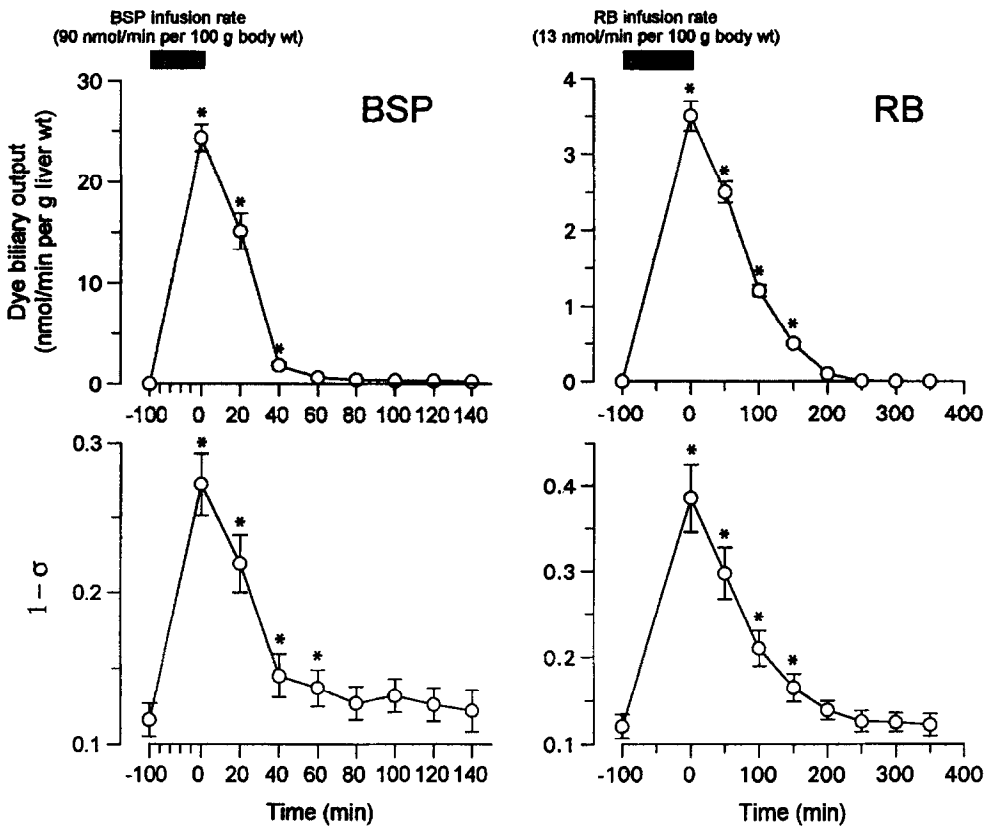

Fig. 3. Changes in biliary dye excretion and the sieving coefficient for [^{14}C]sucrose ($1 - \sigma$) in five animals during administration (100 min) and subsequent interruption of either a BSP infusion of 90 nmol/min per 100 g body wt or an RB infusion of 13 nmol/min per 100 g body wt. Sieving coefficients were calculated by solving Equation 3 (see Materials and Methods), assuming that no change occurred in the diffusion permeability coefficient throughout the experiment (basal values: 0.134 and 0.120 for BSP and RB, respectively). Values are means ± SEM. Key: (*) significantly different from preinfusion values ($P < 0.05$).

the sieving coefficient and/or to changes in the diffusion permeability coefficient. Each of these parameters quantifies a different transport process of the marker, namely diffusion and solvent drag, respectively. Current experimental techniques, unfortunately, do not allow simultaneous quantitation of both processes. As an alternative, changes in biliary permeability may be addressed by evaluating the increments of one of these parameters (i.e. either the sieving coefficient or the diffusion permeability coefficient) necessary to account for the increments in [^{14}C]sucrose bile-to-plasma ratios, assuming that no change has occurred in the other one [4]. For example, by assuming that changes in the [^{14}C]sucrose bile-to-plasma ratio were due only to changes in the sieving coefficient, Equation 3 can be solved for ($1 - \sigma$), k values being assumed to be equal to that in basal conditions. This approach allows changes in biliary [^{14}C]sucrose leakage due to modifications in tight junctional permeability to be distinguished from that due to changes in bile flow.

Statistical analysis

All results are expressed as means ± SEM. Student's paired t-test was used for comparison within groups, and Student's unpaired t-test was used for comparison between groups, after testing the equality of variances with an F test [23]. The level of significance was set at $P < 0.05$. Equation 3 was fitted to the basal experimental data to estimate the initial ($1 - \sigma$) and k values by a least-squares curve-fitting technique. Values of ($1 - \sigma$) or k during dye infusion were obtained by solving Equation 3 with the aid of a mathematical computer program.

Chemicals

[^{14}C]Sucrose was purchased from New England Nuclear (Boston, MA). PR, BCG, BSP, RB and HRP (type II) were obtained from the Sigma Chemical Co. (St. Louis, MO). All the other reagents were of the highest analytical grade available.

RESULTS

The effect of BSP (60 nmol/min per 100 g body wt) on biliary access of both [^{14}C]sucrose and HRP is depicted in Fig. 1. As expected, BSP infusion induced a choleretic effect, which paralleled the increments in the biliary excretion of the dye. Both bile flow and BSP biliary output reached a plateau after 100 min of BSP infusion. Dye administration led to a gradual increase in the [^{14}C]sucrose bile-to-plasma ratio (Fig. 1A and Table 1), producing a progressive displacement toward curves of higher permeability. The [^{14}C]sucrose bile-to-plasma ratio also reached a maximum value after 100 min of dye infusion. On the other hand, in controls, virtually no change in either bile flow or the bile-to-plasma ratio of [^{14}C]sucrose was recorded over the whole experimental

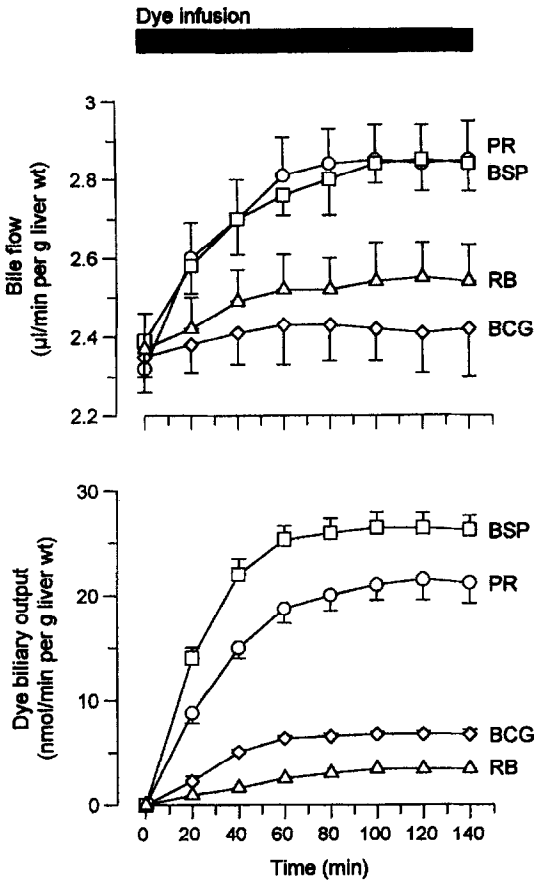

Fig. 4. Changes in bile flow and biliary output of PR (○, N = 5), BCG (◇, N = 5), BSP (□, N = 5) and RB (△, N = 6) following an i.v. infusion of 110, 30, 90 and 13 nmol/min per 100 g body wt, respectively. These infusion rates were chosen so as to attain a similar dye hepatic content at the end of the experiment in the four experimental groups (≈150 nmol/g liver wt). Values are means ± SEM.

period (data not shown). The early (paracellular) peak of the HRP biliary excretion curve was also clearly increased by BSP administration, whereas no change was recorded in the late (transcellular) access of the protein (Fig. 1B and Table 2).

The dependence of this phenomenon on the administered dose was evaluated further, using [^{14}C]sucrose as a probe. Under steady-state conditions of dye excretion, a linear relationship between the increment in the sieving coefficient for the marker and the BSP infused dose could be obtained (Fig. 2), suggesting that the dye increases biliary permeability in a dose-dependent manner. Essentially the same results were obtained when changes in the diffusion permeability coefficient for [^{14}C]sucrose (assuming no change in the sieving coefficient) were plotted. Similarly, a linear dose–effect relationship was apparent when RB was administered (Fig. 2).

Changes in both dye biliary output and the sieving coefficient for [^{14}C]sucrose during administration and subsequent interruption of a steady infusion of BSP and RB are depicted in Fig. 3. Once dye infusion was stopped, the sieving coefficient returned rapidly to the preinfusion value, and virtually the same pattern was discernible when changes in the diffusion permeability coefficient were analyzed. This restoration was linked closely to the decay in the biliary output of the dyes.

The role that dye hydrophobic/hydrophilic balance has in determining the changes in biliary permeability to [^{14}C]sucrose was examined by administrating four different dyes covering a wide range of hydrophobicity. Relative hydrophobicity indices of the individual dyes (obtained according to Equation 1) were as follows: PR = 0, BCG = 0.20, BSP = 0.59 and RB = 1. Figure 4 shows the changes in the bile flow and biliary excretion of the four compounds following i.v. infusions of 110, 30, 90 and 13 nmol/min per 100 g body wt, respectively. These doses were chosen so as to attain a similar dye hepatic content in the four experimental groups at the end of the experiment (PR = 154 ± 13, BCG = 148 ± 17, BSP = 152 ± 4 and RB = 156 ± 12 nmol/g liver wt). At such doses, all dyes increased bile flow. However, none of them influenced biliary excretion of endogenous bile salts (median value during the infusion period: PR = 56.6 ± 3.3, BCG = 54.7 ± 5.0, BSP = 57.3 ± 4.7 and RB = 54.6 ± 4.2 nmol/min per g liver wt). As can be seen in Fig. 5, all dyes increased the [^{14}C]sucrose bile-to-plasma ratio with the exception of PR, which had virtually no effect on this parameter. The changes in the sieving coefficient induced by the dyes under steady-state conditions of dye excretion correlated well with their respective hydrophobicity indices (Fig. 6). Again, the same results were obtained when changes in the permeability diffusion coefficient (assuming no change in the sieving coefficient) were plotted (data not shown). These results suggest that the capability of the cholephilic dyes to increase biliary permeability to sucrose depends on its hydrophobic/hydrophilic balance.

DISCUSSION

The present study was undertaken to determine whether cholephilic dyes modify tight junctional permeability in the rat. To achieve this goal, we analyzed the effect of the model cholephilic dye BSP on the access into bile of two permeability probes, [^{14}C]sucrose and HRP. The former served as a continuous monitor of the paracellular biliary access [3, 4, 13, 14], whereas the latter was employed as an indicator of both paracellular and transcellular biliary access of the marker [15]. It should be noted, however, that although commonly used to evaluate biliary permeability, both methods have some shortcomings. A minority, microtubule-dependent transcytotic pathway for the entry of sucrose into the canaliculus has been claimed [24–26], thus introducing uncertainty as to whether this route is involved in the enhanced biliary access of the marker. Likewise, a small contribution of a rapid, microtubule-independent transcellular access of HRP to the early peak of HRP biliary excretion curve was described previously [27]. However, the fact that concordant conclusions could be drawn even though two independent methods were employed allows us to suppose that methodological artifacts probably do not account for our results. Furthermore, transcellular biliary access of HRP, which like that of sucrose occurs predominantly by fluid-phase endocytosis [28], was not altered significantly by BSP administration (see Fig. 1B and Table 2). Therefore, the supposition of a contribution of this pathway to the increase in the [^{14}C]sucrose bile-to-plasma ratios could be discarded.

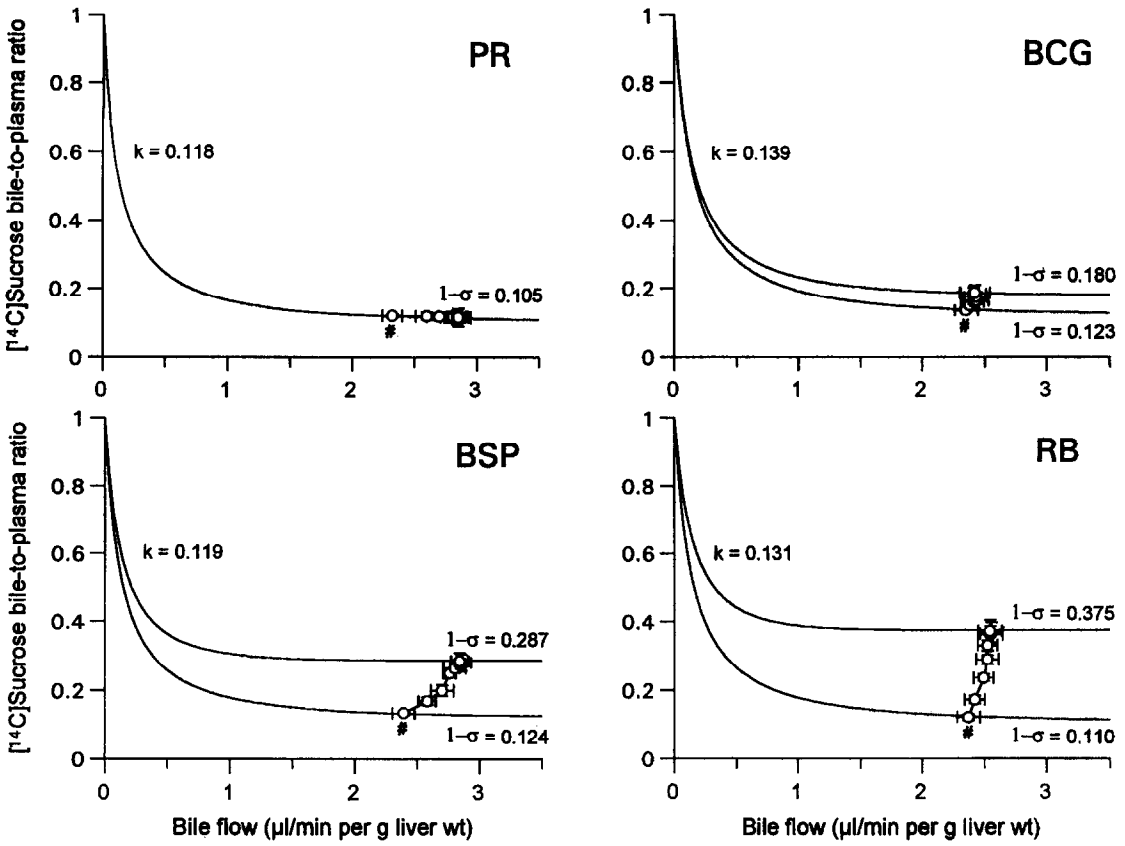

Fig. 5. Relation between [^{14}C]sucrose bile-to-plasma ratio and bile flow in animals given infusions of 110, 30, 90 and 13 nmol/min per 100 g body wt of PR (N = 5), BCG (N = 5), BSP (N = 5) and RB (N = 6), respectively. Following a basal collection period, cholephilic dyes were infused, and blood and bile samples were collected in seven consecutive periods; basal values are denoted with a number symbol (#). Bottom curves were obtained by fitting basal [^{14}C]sucrose bile-to-plasma ratio and bile flow values to Equation 3 (see Materials and Methods). Upper curves were obtained by fitting the [^{14}C]sucrose bile-to-plasma ratio and the bile flow recorded during the last (120–140 min) bile collection period to the same equation, assuming that no change occurred in the diffusion permeability coefficient (k) throughout the experiment. Values are means ± SEM.

The results of this study suggest that cholephilic dyes increase biliary permeability in a reversible and dose-dependent manner, and that this effect depends on the hydrophobic/hydrophilic balance of the dye. This phenomenon was observed even though low, choleretic doses of the dyes were administered. Such doses were far below those needed either to reach the maximal biliary secretory rate or to influence biliary excretion of endogenous bile salts. This excludes the possibility that the origin of the changes observed could be due to overt forms of hepatotoxicity or to intrahepatic accumulation of bile acids.

The precise site and mechanism by which cholephilic dyes increase biliary permeability cannot be determined from this study. However, our results raise some points about the possible action mechanisms.

Tight junctional permeability is influenced by a number of physical factors, including osmotic and hydrostatic pressure [29]. Following canalicular excretion of osmotically active compounds, osmotic gradients are created and biliary pressure is elevated by the resultant choleresis [30]. By acting in concert, therefore, it is conceivable that both factors may increase tight junctional permeability during cholephilic dye-induced choleresis, as was proposed previously for bile salts [3]. Our results, however, are clearly not in line with this possibility,

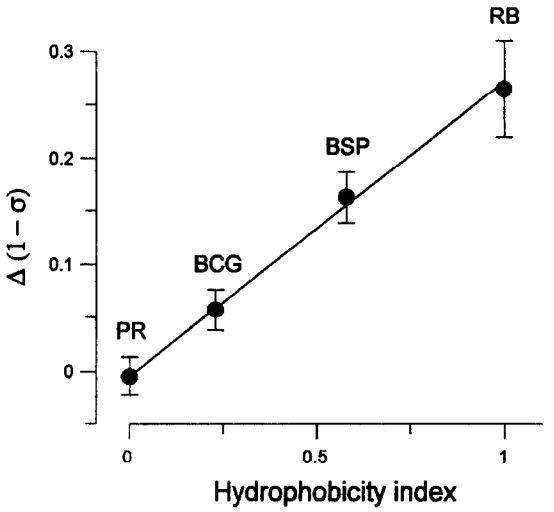

Fig. 6. Dependency of the changes in the sieving coefficient for [^{14}C]sucrose (1 − σ) shown in Fig. 4 on the hydrophobicity index of the infused dyes, calculated according to Equation 1 (see Materials and Methods). Final sieving coefficients were obtained by solving Equation 3 (see Materials and Methods) using the [^{14}C]sucrose bile-to-plasma ratio and bile flow values obtained during the last (120–140 min) bile collection period and assuming that no change occurred in the diffusion permeability coefficient during dye infusion. Values are means ± SEM, N = 5–6.

since no correlation could be obtained between the changes in biliary permeability induced by each dye and its respective biliary outputs or choleretic effects. This concept became evident from experiments in which PR was administered, but no effect on biliary permeability was apparent despite both dye biliary output and bile flow reaching comparatively high values. Thus, the observed effects appear to be more in response to a specific effect of the dyes than to canalicular choleresis itself.

Recently, different intracellular mechanisms have been proposed to regulate tight junctional permeability in the liver. Elevated intracellular Ca^{2+} concentrations have been reported to increase biliary permeability, presumably through mechanisms involving Ca^{2+}-dependent kinases and/or Ca^{2+}-dependent contraction of microfilaments [6, 31, 32]. Interestingly, cholephilic dyes were shown to inhibit hepatic mitochondrial oxidative phosphorylation [33, 34], and chemical hypoxia was described to increase hepatocellular Ca^{2+} [35]. The involvement of this mechanism, however, must remain speculative since, as far as we know, neither direct measurement of intracellular Ca^{2+} levels nor the onset of mitochondrial dysfunction following dye administration has been established. In addition, a direct effect on either hepatocellular tight junctions or microfilaments, whose normal functioning was shown to be necessary to preserve both morphological and functional integrity of junctional structures [36], should be considered. In turn, direct interaction of the dyes with junctional structures, if present, may occur from both intrahepatocytic or intracanalicular sites. Whichever is the case, hydrophobic interactions appear to be involved, as suggested by the partial dependence of the observed effects on dye lipophilicity. This is not surprising since interactions of this nature are presumed to be important in determining association of dyes to subcellular structures [37].

In summary, this study provides the first evidence that cholephilic dyes increase tight junctional permeability in the rat and that dye hydrophobic/hydrophilic balance would be a key factor governing this effect.

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