Ursodeoxycholate Stimulates Na\textsuperscript{+}-H\textsuperscript{+} Exchange in Rat Liver Basolateral Plasma Membrane Vesicles

Richard H. Moseley, Nazzareno Ballatori, Daniel J. Smith, and James L. Boyer
Department of Medicine, University of Michigan School of Medicine, Ann Arbor, Michigan, 48105; and Liver Center, Yale University School of Medicine, New Haven, Connecticut 06510

Abstract

Na\textsuperscript{+}:H\textsuperscript{+} and Cl\textsuperscript{-}:HCO\textsubscript{3}{-} exchange are localized, respectively, to basolateral (bLPM) and canalicular (cLPM) rat liver plasma membranes. To determine whether these exchangers play a role in bile formation, we examined the effect of a choletheric agent, ursodeoxycholate (UDCA), on these exchange mechanisms. \textsuperscript{22}Na (1 mM) and \textsuperscript{36}Cl (5 mM) uptake was determined using outwardly directed H\textsuperscript{+} and HCO\textsubscript{3}{-} gradients, respectively. Preincubation of bLPM vesicles with UDCA (0-500 \mu M) resulted in a concentration-dependent increase in initial rates of amiloride-sensitive pH-driven \textsuperscript{22}Na\textsuperscript{+} uptake, with a maximal effect at 200 \mu M. UDCA (200 \mu M) increased \( V_{\text{max}} \) from 23±2 (control) to 37±7 nmol/min per mg protein; apparent \( K_{\text{a}} \) for \textsuperscript{22}Na\textsuperscript{+} was unchanged. Preincubation with tauroursodeoxycholate (200 \mu M), taurocholate (10-200 \mu M) or cholate, chenodeoxycholate, or deoxycholate (200 \mu M) had no effect on pH-driven \textsuperscript{22}Na\textsuperscript{+} uptake. UDCA (200 \mu M) had no effect on either membrane lipid fluidity, assessed by steady-state fluorescence polarization using the probes 1,6-diphenyl-1,3,5-hexatriene, 12-(9-anthroyloxy) stearic acid, and 2-(9-anthroyloxy) stearic acid (2-AS), or Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity in bLPM vesicles. In cLPM vesicles, UDCA (0-500 \mu M) had no stimulatory effect on initial rates of HCO\textsubscript{3}{-}-driven Cl\textsuperscript{-} uptake. Enhanced basolateral Na\textsuperscript{+}:H\textsuperscript{+} exchange activity, leading to intracellular HCO\textsubscript{3}{-} concentrations above equilibrium, may account for the bicarbonate-rich choleresis after UDCA infusion.

Introduction

Ursodeoxycholate (UDCA),\textsuperscript{1} a 3α,7β dihydroxy bile acid that is increasingly used to dissolve cholesterol gallstones in man, induces a marked choletheric response in rats that is associated with a selective increase in canalicul bicarbonate secretion.


Address correspondence to Richard H. Moseley, M.D., Gastroenterology Division, Department of Medicine, Veterans Administration Medical Center, 2215 Fuller Road, Ann Arbor, MI 48105.

Received for publication 13 March 1986 and in revised form 2 April 1987.

1. Abbreviations used in this paper: 2-AS, 2-(9-anthroyloxy) stearic acid; 12-AS, 12-(9-anthroyloxy) stearic acid; bLPM, basolateral liver plasma membrane vesicle; cLPM, canalicular liver plasma membrane vesicle; DPH, 1,6-diphenyl-1,3,5-hexatriene; MES, 2(N-morpholino)ethanesulfonic acid; TC, taurocholate; TUDCA, tauroursodeoxycholate; UDCA, ursodeoxycholate.

The Journal of Clinical Investigation, Inc.
Volume 80, September 1987, 684–690

(1, 2). This property, as well as the observation that bicarbonate is required for the maintenance of bile acid-independent bile formation in the isolated perfused rat liver model (3–5), suggests that bicarbonate transport may be important in the generation of hepatic bile flow. Preliminary observations in the fluorocarbon-perfused rat liver also indicate that UDCA-stimulated choleresis is dependent on sodium and can be inhibited by amiloride, a competitive inhibitor of Na\textsuperscript{+}:H\textsuperscript{+} exchange, and analogues of amiloride (6, 7). In addition, we have recently identified and characterized basolateral Na\textsuperscript{+}:H\textsuperscript{+} and canalicular Cl\textsuperscript{-}:HCO\textsubscript{3}{-} exchange on these respective domains of the hepatocyte plasma membrane (8, 9). Altogether, this evidence, although circumstantial, is consistent with a role for Na\textsuperscript{+}:H\textsuperscript{+} and Cl\textsuperscript{-}:HCO\textsubscript{3}{-} exchange in bile formation, as discussed in recent reviews (10, 11). It is therefore possible that bile acids such as UDCA might exert their actions via direct effects on these ion exchange mechanisms. To further explore the role of membrane transport processes in this critical hepatocellular function, we examined the effects of UDCA on Na\textsuperscript{+}:H\textsuperscript{+} and Cl\textsuperscript{-}:HCO\textsubscript{3}{-} exchange activities in purified rat liver basolateral (bLPM) and canalicular (cLPM) plasma membrane vesicles. The effects on Na\textsuperscript{+}:H\textsuperscript{+} exchange activity of the structurally related tauro conjugate, tauroursodeoxycholate (TUDCA), as well as the bile acids, taurocholate (TC), cholate, deoxycholate, and chenodeoxycholate were also examined.

Methods

Materials. \textsuperscript{22}Na was obtained from Amersham Corp., Arlington Heights, IL. \textsuperscript{36}Cl was obtained as a 0.2–3.0 M HCl solution from New England Nuclear, Boston, MA, and was neutralized with tetramethylammonium hydroxide to a pH of 7.5 before use. Amiloride was a gift from Merck, Sharp and Dohme Research Laboratories (West Point, PA). UDCA and TUDCA were generously provided by Dr. Kenichi Kitani and were obtained from Tokyo Tanabe Co., Tokyo, Japan. Concentrated stock solutions of UDCA and TUDCA were prepared in propylene glycol, and equivalent volumes of propylene glycol were added to all control incubations. All other chemicals and reagents were obtained from Sigma Chemical Co., St. Louis, MO. All water used in preparing media was deionized and filtered through 0.45-μm Millipore filters. Valinomycin was stored in 95% ethanol.

Animals. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Kingston, NY) weighing 200–250 g were used for all studies. The animals had free access to water and Purina rodent chow and were housed in a constant temperature-humidity environment with alternating 12-h light and dark cycles. Fed animals were routinely sacrificed by decapitation between 7:30 and 8:30 AM.

Isolation of cLPM and bLPM. The method for isolation of the cLPM and bLPM as well as their biochemical and morphologic characterization have been described in detail (8, 12). A virtually complete separation of bLPM and cLPM by this method is reflected by the absence in cLPM of Na\textsuperscript{+},K\textsuperscript{+}-ATPase, glucagon-stimulatable adenylate cyclase, and intact secretory component (12). By freeze fracture analysis, ~80% of cLPM and 73% of bLPM vesicles exhibit right-side-out configuration (8, 12).
Vesicles were preloaded with desired media by suspending freshly isolated membrane vesicles by tight homogenization (10 up-and-down strokes, type B Dounce homogenizer [Kontes Glass Co., Vineland, NJ]) in membrane resuspension media as defined in the figure legends. Vesicles were then frozen and stored in liquid nitrogen (protein concentration > 5 mg/ml) before use.

Before transport studies, the frozen vesicle suspensions were quickly thawed in a 37°C water bath, diluted to the desired protein concentration, and passed repeatedly (15±) through a 25-gauge needle.

**2Na and 36Cl uptake determination.** For measurements of 2Na and 36Cl uptake, 20 µl (100–130 µg protein) of the membrane vesicle suspension was added at 25°C to 80 µl reaction mixture containing the radiolabeled material plus the appropriate medium. Uptake was terminated at specific time intervals by the addition of 3 ml of ice-cold stop solution consisting of 204 mM sucrose, 150 mM K gluconate, 0.2 mM Ca gluconate, 5 mM Mg gluconate, and 10 mM Hepes/Tris, pH 7.5. Membrane vesicle-associated ligand was separated from free ligand by immediate and rapid filtration through a 0.45-µm Millipore filter (HAWP). The filter was washed twice with 3 ml stop solution, dissolved in Redisol VP (Beckman Instruments, Inc., Palo Alto, CA) and counted in a Beckman LS 2000 liquid scintillation counter. Non-specific binding of isotope to filter and membrane vesicles was determined in each experiment by addition, at 0-4°C, of reaction mixture and stop solution to 20 µl membrane suspension. This membrane/filter blank was subtracted from all determinations. Because of the presence of an electrogenic component to Na+ uptake in blLPM vesicles (8, 13), all studies were performed under voltage-clamp conditions, in which the contribution of the membrane potential to the observed pH gradient-dependent Na+ uptake is minimized by the addition of the K+ ionophore, valinomycin (5 µg/mg protein) in the presence of 100 mM K+ on both sides of the vesicle membrane. Under these conditions, amiloride-sensitive pH-driven Na+ uptake (attributable to Na+:H+ exchange) is preferentially studied. To assess the effect of various bile acids on initial rates of pH-stimulated Na+ uptake, uptake was determined at 15 s. For experiments requiring preincubation of bile acids with membrane vesicles, concentrated solutions of bile acids in propylene glycol were used and equivalent amounts of propylene glycol served as control. Preliminary studies utilizing the pH-sensitive dye, acridine orange, demonstrated no effect of bile acids or propylene glycol on the rate of dissipation of the imposed pH gradient in blLPM vesicles.

Unless otherwise indicated, all experimental data were obtained from triplicate analysis of three or more separate membrane preparations. All values are expressed as mean±SE. The data were compared by Student's t test; differences were considered to be statistically significant when P < 0.05.

**Na+,K+-ATPase assays.** LPM preparations were assayed for Na+,K+-ATPase activity using a previously described coupled enzyme assay in which the production of ADP by the ATPase reaction is linked to NADH oxidation using pyruvate kinase and lactate dehydrogenase as coupling enzymes (14).

**Fluorescence polarization measurements.** Fluorescence polarization was studied with a SLM 8000 photon-counting spectrofluorometer (SLM Instruments, Inc., Urbana, IL) using 1,6-diphenyl-1,3,5-hexatriene (DPH; Eastman Kodak Co., Rochester, NY) and 12-(9-anthroyloxy) stearic acid (12-AS; Molecular Probes, Inc., Junction City, OR) as probes for the hydrophobic core of the membrane (15), and 2-(9-anthroyloxy) stearic acid (2-AS; Molecular Probes, Inc.) as a probe for the surface of the membrane. For insertion into the membrane, an aliquot of probe was first dried on the inner surface of a test tube, an aliquot of membrane suspension added, and the tube vortexed. Measurements were performed as described by Lakowicz (16), and anisotropy (r) calculated using Perrin's equation:

\[ r = (I_0 - I) / (I_0 + 2I) \]

where I0 and I are the intensities of the fluorescence signal in the presence of probe and therefore was discounted in these experiments.

### Results

The effect of UDCA on pH-driven Na+ transport was first studied under conditions in which the bile acid was present only in the extravesicular medium. As shown in Table I, no effect on Na+ uptake was observed under these conditions. However, when membrane suspensions were preincubated with UDCA at 25°C for 10 min, a concentration-dependent increase in pH-driven Na+ uptake was observed with a maximal effect at 200 µM UDCA (Fig. 1). In subsequent experiments (data not shown), this requirement for preincubation was confirmed with no stimulation observed after 1 min preincubation, but enhancement of pH-driven Na+ uptake at 5 min was similar to that shown at 10 min. Preincubation of membrane vesicles with UDCA had no effect on the degree of uptake at equilibrium.

To determine if UDCA was selective in increasing Na+:H+ exchange activity, we next examined the effects of the structurally related taurine conjugate of UDCA, TUDCA, and TC on pH-driven Na+ uptake in blLPM. As shown in Fig. 2, TUDCA and TC did not significantly increase pH-driven Na+ uptake above control levels, as compared with UDCA. Additionally, the similar inhibition by amiloride, a competitive inhibitor of Na+:H+ exchange (17), of pH-driven Na+ uptake, regardless of the bile acid studied, is consistent with a selective enhancement of Na+:H+ exchange by UDCA. The selective nature of the observed enhancement of Na+:H+ exchange activity by UDCA is suggested even further by studies with other bile acids. As shown in Table II, neither cholate, deoxycholate, nor chenodeoxycholate significantly increased pH-driven Na+ uptake above control levels, as compared with UDCA.

The component of Na+ flux in blLPM vesicles that is observed under voltage clamp conditions has been previously attributed to amiloride-sensitive Na+:H+ exchange (8). Never-

### Table I. Lack of Effect of UDCA on pH-driven Na+ Uptake in blLPM Vesicles without Preincubation

<table>
<thead>
<tr>
<th>15-s uptake values (pmol/mg protein)</th>
<th>Control</th>
<th>25 µM UDCA</th>
<th>50 µM UDCA</th>
<th>100 µM UDCA</th>
<th>200 µM UDCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>232±48</td>
<td>243±50</td>
<td>204±59</td>
<td>217±31</td>
<td>233±49</td>
</tr>
</tbody>
</table>

Vesicles were preloaded with a pH 5.9 buffer (succrose, 82 mM; 2(N-morpholinio)-ethanesulfonic acid (MES), 91 mM; Tris, 29 mM; Hepes, 14 mM; K gluconate, 100 mM; and Ca gluconate, 0.2 mM) and treated with valinomycin (5 µg/mg protein). Initial rates of sodium uptake (1 mM) were determined at 25°C by diluting vesicles into reaction medium of pH 7.9 (succrose, 70 mM; Hepes, 76 mM; Tris, 70 mM; K gluconate, 100 mM; Ca gluconate, 0.2 mM) containing 2Na+ with and without varying concentrations of UDCA. Data represent the mean±SE of triplicate analysis of three separate membrane preparations. UDCA had no effect on Na+:H+ exchange under these conditions (P = NS).
Nevertheless, to study whether UDCA alters Na⁺ permeability in bLPM vesicles, Na⁺ flux was measured under pH-equilibrated conditions in the presence and absence of UDCA. As illustrated in Fig. 3, at a concentration and under preincubation conditions that result in maximal enhancement of pH-driven Na⁺ uptake, UDCA had no effect on Na⁺ flux.

### Table II. Effect of Bile Acids on pH-driven Na⁺ Uptake in bLPM Vesicles

<table>
<thead>
<tr>
<th>Condition</th>
<th>15-s uptake value (pmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>266±36</td>
</tr>
<tr>
<td>200 μM UDCA</td>
<td>353±26*</td>
</tr>
<tr>
<td>200 μM deoxycholate</td>
<td>316±22</td>
</tr>
<tr>
<td>200 μM chenodeoxycholate</td>
<td>295±19</td>
</tr>
<tr>
<td>200 μM cholate</td>
<td>276±40</td>
</tr>
</tbody>
</table>

Vesicles, preloaded with a pH 5.9 buffer (sucrose, 82 mM; MES, 91 mM; Tris, 29 mM; Hepes, 14 mM; K gluconate, 100 mM; Ca gluconate, 0.2 mM) and treated with valinomycin (5 μg/mg protein) were preincubated with designated concentrations of bile acids for 10 min at 25°C. 15-s uptakes of Na⁺ (1 mM) were then determined, at 25°C, by diluting vesicles fivefold into reaction medium of pH 7.9 containing 22Na (sucrose, 70 mM; Hepes, 76 mM; Tris, 70 mM; K⁺ gluconate, 100 mM; Ca gluconate, 0.2 mM) and appropriate concentrations of UDCA. Data represents the mean±SE of triplicate analysis from at least three separate membrane preparations (range, 3–5 membrane preparations). Only UDCA resulted in a significant increase in pH-driven Na⁺ uptake above control.

* *P < 0.05.

In contrast to the observed enhancement of Na⁺:H⁺ exchange in bLPM vesicles, under the same optimal preincubation conditions, UDCA did not have any stimulatory effect on Cl⁻:HCO₃⁻ exchange in cLPM vesicles. As shown in Fig. 4, preincubation of membrane vesicles with either 200 μM UDCA or 200 μM TUDCA did not increase HCO₃⁻ gradient-driven Cl⁻ uptake above control values. In fact, a small degree of cis-inhibition of HCO₃⁻ gradient-driven Cl⁻ uptake resulted from preincubation with either bile acid. Similar results were obtained at both lower (50, 100 μM) and higher (400, 500 μM) concentrations of UDCA, at various preincubation times as well as in the absence of any preincubation (Table III).

Recent findings in rat colonic brush border membrane vesicles (18) suggest that membrane lipid fluidity may exert a
modulatory effect on Na"+H"+ exchange activity. To determine whether the enhancement of Na"+H"+ exchange activity observed with UDCA was the result of an alteration in lipid fluidity, we assessed the effects of UDCA on membrane lipid fluidity after appropriate preincubations by steady-state fluorescence polarization using the probes DPH, 12-AS, and 2-AS. At a concentration of UDCA that resulted in a 32% increase over control in pH-driven Na"+ uptake (mean of four separate membrane preparations), no effect on anisotropy of the fluorescent probes was observed (Table IV).

TC, as well as taurochenodeoxycholate and deoxycholate, cause an immediate and reversible concentration-dependent inhibition of Na"+,K"+-ATPase activity in isolated rat liver plasma membranes (19). To determine the degree of specificity of UDCA-induced stimulation of Na"+:H"+ exchange activity in bLPM, the effect of UDCA on Na"+,K"+-ATPase activity in isolated membrane vesicles was examined. As illustrated in Table V, incubation of plasma membranes with UDCA had no significant effect on Na"+,K"+-ATPase activity.

Finally, the effect of increasing extravascular concentrations of Na"+ on the initial rates of pH-driven Na"+ uptake under control and UDCA-stimulated conditions was studied. pH-stimulated Na"+ uptake was enhanced by UDCA (200 μM) at all external Na"+ concentrations studied (Table VI). Regres-

Table III. Effect of UDCA on Cl"-HCO3" Exchange in cLPM Vesicles (15-s Uptake Values)

<table>
<thead>
<tr>
<th>Preincubation duration</th>
<th>0 min</th>
<th>5 min</th>
<th>10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>nmol/mg protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.7±0.2</td>
<td>4.5±0.1</td>
<td>—</td>
</tr>
<tr>
<td>50 μM UDCA</td>
<td>3.9±0.2*</td>
<td>3.6±0.2*</td>
<td>3.7±0.1</td>
</tr>
<tr>
<td>100 μM UDCA</td>
<td>4.1±0.1*</td>
<td>3.7±0.1*</td>
<td>3.3±0.2</td>
</tr>
<tr>
<td>200 μM UDCA</td>
<td>3.4±0.2*</td>
<td>3.5±0.2*</td>
<td>3.4±0.3</td>
</tr>
<tr>
<td>400 μM UDCA</td>
<td>4.0±0.3</td>
<td>3.5±0.3*</td>
<td>3.2±0.4</td>
</tr>
<tr>
<td>500 μM UDCA</td>
<td>3.8±0.3*</td>
<td>3.1±0.3*</td>
<td>3.0±0.1</td>
</tr>
</tbody>
</table>

Vesicles were loaded in a pH 7.7 buffer containing 50 mM HCO3" (150 mM, sucrose; 100 mM, Tris HCO3; 50 mM, choline bicarbonate; 35 mM, Tris; 35 mM, Hepes; 0.2 mM, Ca gluconate; and 5 mM, Mg gluconate) and, where noted, incubated in equivalent amounts of propylene glycol (control) and UDCA in propylene glycol, at 25°C. Initial rates (15 s) of chloride (5 mM) uptake were determined as in Fig. 4. Data represents the mean±SE of triplicate analysis of three separate membrane vesicle preparations. * P < 0.05.

Table IV. Effect of UDCA on Anisotropy (r) of Fluorescent Probes in Basolateral Membrane Vesicles

<table>
<thead>
<tr>
<th>Control</th>
<th>Propylene glycol</th>
<th>UDCA</th>
<th>TUDCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 μM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPH</td>
<td>0.225±0.003</td>
<td>0.225±0.003</td>
<td>0.225±0.003</td>
</tr>
<tr>
<td>12-AS</td>
<td>0.062±0.003</td>
<td>0.062±0.003</td>
<td>0.062±0.003</td>
</tr>
<tr>
<td>2-AS</td>
<td>0.102±0.003</td>
<td>0.102±0.003</td>
<td>0.102±0.003</td>
</tr>
</tbody>
</table>

Data represent the mean±SD of nine readings from each of three separate membrane preparations. UDCA had no effect on anisotropy of the fluorescent probes.

Ursodeoxycholate-induced Stimulation of Na"+-H"+ Exchange
UDCA had no effect on Na+,K+-ATPase activity, under conditions similar to those in Fig. 1, at increasing extravesicular concentrations of Na+ gluconate. 15-s uptakes were determined. The data represent the mean±SE of triplicate analysis from three separate membrane preparations.

Table V. Effect of UDCA on Na+,K+-ATPase Activity of Liver Plasma Membrane Preparation

<table>
<thead>
<tr>
<th>Control</th>
<th>Propylene glycol</th>
<th>UDCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na+,K+-ATPase activity (μmol Pi/mg·h)</td>
<td>16.1±2.3</td>
<td>16.9±4.4</td>
</tr>
</tbody>
</table>

Data represent the mean±SD from five separate membrane preparations. UDCA had no effect on Na+,K+-ATPase activity.

Discussion

The bicarbonate-rich choleresis observed after UDCA infusion provides a unique model for investigating the potential cellular mechanisms responsible for bile formation. Using liver plasma membrane vesicles, the present studies provide direct evidence for a stimulatory effect of UDCA on basolateral membrane Na+/H+ exchange. This evidence includes (a) a concentration-dependent enhancement of amiloride-sensitive pH gradient-driven Na+ uptake in basolateral membrane vesicles preincubated with Ursodeoxycholate, (b) no enhancement of pH gradient-driven Na+ uptake with the less choleretic (20), structurally related taurine conjugate of UDCA, or with TC, cholate, chenodeoxycholate and deoxycholate, and (c) no effect of UDCA on Na+ flux under pH-equilibrated conditions, Na+,K+-ATPase activity, or membrane lipid fluidity, as assessed by steady-state fluorescence polarization.

If stimulation of Na+:H+ exchange activity is to be observed, UDCA must be preincubated with membrane vesicles for up to 10 min, suggesting that UDCA does not interact with the exchanger on the outer membrane surface. Because the majority of membrane vesicles exhibit right-side-out configuration, the site of interaction may be localized to the inner aspect of the membrane. Uptake of UDCA into the vesicle interior might then be necessary for its effect to be observed. A similar mechanism, in which a bile salt might modify ion movement by accumulating within hepatocytes, was recently proposed to explain the hypercholeretic effect of UDCA when administered to the intact animal (21). The exact mechanism by which UDCA is transported into the hepatocyte is not known, although conjugation may be the rate-limiting step in its overall transport from plasma to bile (22). Uptake of the dihydroxy unconjugated UDCA might be expected to proceed primarily via a nonsaturable sodium-independent process, given the finding that sodium-dependent, ouabain-suppressible uptake of bile acids in cultured rat hepatocytes is associated with increased ring hydroxylation and amino acid conjugation (23). Preliminary observations in isolated rat hepatocytes support this contention (24).

The kinetic studies, demonstrating an increase in Vmax without an alteration in Km for Na+, combined with the absence of changes in membrane lipid fluidity, suggest that UDCA may result in a minor allosteric modification of the exchanger that increases its efficiency or its turnover. Such an effect would be similar to the modulating effects of intracellular protons on Na+:H+ exchange (25). Indeed, UDCA uptake into hepatocytes has been associated with intracellular acidification (24). Because kinetic features were studied in the presence of a steep pH gradient, the observed effect of UDCA is more likely to be the result of increased turnover of Na+:H+ exchange rather than a change in the modifier site. In fact, it is unlikely that the effect of UDCA on Na+:H+ exchange in membrane vesicles in the present study is directly related to intravesicular acidification and changes in the imposed pH gradient, because preincubation with bile acids with similar pK's to UDCA (cholate, chenodeoxycholate) did not increase initial rates of pH-driven Na+ uptake (Table II). In addition, the high buffering capacity of the intravesicular media (140 mM Tris, Hepes, and MES) make significant changes in intravesicular pH unlikely. Parenthetically, in view of the high extracellular concentration of Na+ in vivo, it is not surprising that modulation of the exchanger does not occur through changes in Km.

Na+:H+ exchange could also be affected by changes in its membrane lipid environment, and recent work in the intestine has noted this relationship (17, 26). The absence of appreciable changes in membrane lipid fluidity rules against this effect in the present study. In addition, lack of an effect on the Na+,K+-ATPase activity at UDCA concentrations which result in maximal stimulation of Na+:H+ exchange differs somewhat from previous studies that demonstrated both inhibitory and stimulatory effects of bile salts on plasma membrane Na+,K+-ATPase activity or fluidity (18, 27). However, higher concentrations of bile salts were used in these reports. The findings in the present study suggest that the effects of UDCA cannot be ascribed to nonspecific effects on plasma membrane fluidity and enzyme activity.

In CLPM vesicles, neither TUDCA nor UDCA had a direct stimulatory effect on Cl−:HCO3− exchange. In fact, UDCA...
(0–500 μM) and TUDCA (200 μM) exerted a small but significant inhibition of HCO$_3^-$ gradient-driven Cl$^-$ uptake into cLPM vesicles (Fig. 4 and Table III). This cis-inhibition by UDCA suggests the existence of an organic anion exchanger on the canalicular membrane, the presence of which was previously undetectable (9). Clearly, additional studies are required to address this question and determine if the canalicular membrane of the rat liver contains an anion exchange transport system similar to that recently described for bile acids in rat intestinal basolateral membrane vesicles (28).

The specificity of the stimulation of Na$^+$/H$^+$ exchange by UDCA suggests that this membrane antiport could play a role in the choleretic observed with this bile acid and lends further support to a proposed model of bile formation (8, 9, 11) in which basolateral Na$^+$/H$^+$ exchange, in conjunction with cyto- 

solic carbonic anhydrase, acts as a driving force for the can-

alicular secretion of HCO$_3^-$ via canalicular Cl$^-$/HCO$_3^-$ exchange. This model predicts that the activity of the Na$^+$/H$^+$ exchanger will generate intracellular OH$^-$ (and in turn, HCO$_3^-$), and therefore raise intracellular HCO$_3^-$ above its equilibrium concentration. The net secretion of HCO$_3^-$, with Na$^+$ entering the canalculus passively via the paracellular pathway, would increase the osmotic activity of bile and thereby oblige the secretion of water and possibly of other electrolytes (8). Therefore, agents that enhance Na$^+$/H$^+$ exchange should increase canalicular HCO$_3^-$ secretion and bile flow, whereas agents that inhibit Na$^+$/H$^+$ exchange should result in intracellular HCO$_3^-$ concentrations closer to equilibrium concentration, and decrease canalicular HCO$_3^-$ secretion and diminish bile flow.

To date, however, no direct evidence has been obtained to confirm a role for Na$^+$/H$^+$ exchange in bile formation in contrast to other diverse cellular functions, including intracellular pH control, cell volume regulation, and cell growth and proliferation (11, 29, 30). Nevertheless, several additional observations with known cholestatic and choleretic agents support this model. Amiloride and amiloride analogues significantly di-

minish UDCA-stimulated bile flow and bicarbonate output in perfused rat livers (6, 7) although a primary effect on basal bile flow is less clear (31). The carbonic anhydrase inhibitor, acet-

azolamide, has also been shown to decrease both UDCA-stim-

ulated bile flow and biliary bicarbonate concentration in similar preparations (32). Ethynyl estradiol, a potent cholestatic agent, produced almost complete inhibition of Na$^+$/H$^+$ ex-

change activity in rat liver sinusoidal membrane vesicles (33). Finally, both glucocorticoids and thyroid hormone, known choleretic agents in vivo (34, 35), stimulate Na$^+$/H$^+$ exchange in other epithelia (36, 37). Whereas caution is warranted in ascribing physiologic relevance to these observations, the findings in the present study demonstrate that UDCA, a known choleretic agent, can stimulate Na$^+$/H$^+$ exchange in vitro at bile salt concentrations within the range reported for both liver tissue and cultured hepatocytes after UDCA administration (38, 39), and well below the critical micellar concentration of UDCA.

In conclusion, preincubation of isolated basolateral mem-

brane vesicles with ursodeoxycholate results in a concentra-

tion-dependent increase in amiloride-sensitive Na$^+$/H$^+$ exchange. These findings have been discussed primarily with respect to an evolving model of bile formation, in which secondary active Na$^+$/H$^+$ exchange acts as a driving force for the net canalicular secretion of HCO$_3^-$. However, the physiologic significance of these in vitro findings must eventually depend on in vivo studies that can more directly establish a role for or against electrolyte transport in bile formation, such as isolated hepatocyte couplets (40). A recent report that ursodeoxycholy-

late-induced choleresis in the isolated perfused rat liver is not dependent on bicarbonate per se but is the result of H$^+$ (or OH$^-$) transport (41) supports this interpretation.

Acknowledgments

We thank Carolyn Barrett and Steven Murphy for excellent technical assistance, John W. Dobbins for helpful discussions, and E. Brooks Comstock for efficient typing of the manuscript. This work was supported in part by grants AM-07356, AM-31493, AM-34989, and AM-07616 from the National Institutes of Health. Dr. Moseley was a recipient of an American Liver Foundation Postdoctoral Research Fellowship Award.

References


