Role of sodium/hydrogen exchanger isoform NHE3 in fluid secretion and absorption in mouse and rat cholangiocytes

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Mennone, Albert, Daniel Biemesderfer, Daniel Negioianu, Chao-Ling Yang, Thecla Abbiati, Patrick J. Schultheis, Gary E. Shull, Peter S. Aronson, and James L. Boyer. Role of sodium/hydrogen exchanger isoform NHE3 in fluid secretion and absorption in mouse and rat cholangiocytes. Am J Physiol Gastrointest Liver Physiol 280: G247–G254, 2001.—Na+/H+ exchanger (NHE) isoforms play important roles in intracellular pH regulation and in fluid absorption. The isoform NHE3 has been localized to apical surfaces of epithelia and in some tissues may facilitate the absorption of NaCl. To determine whether the apical isoform NHE3 is present in cholangiocytes and to examine whether it has a functional role in cholangiocyte fluid secretion and absorption, immunocytochemical studies were performed in rat liver with NHE3 antibodies and functional studies were obtained in isolated bile duct units from wild-type and NHE3 (−/−) mice after stimulation with forskolin, using video-microscopic techniques. Our results indicate that NHE3 protein is present on the apical membranes of rat cholangiocytes and on the canalicular membrane of hepatocytes. Western blots also detect NHE3 protein in rat cholangiocytes and isolated canalicular membranes. After stimulation with forskolin, duct units from NHE3 (−/−) mice fail to absorb the secreted fluid from the cholangiocyte lumen compared with control animals. Similar findings were observed in isolated bile duct units from wild-type mice and rats in the presence of the Na+/H+ exchanger inhibitor 5-(N-ethyl-N-isopropyl)amiloride. In contrast, we could not demonstrate absorption of fluid from the canalicular lumen of mouse or rat hepatocyte couplets after stimulation of secretion with forskolin. These findings indicate that NHE3 is located on the apical membrane of rat cholangiocytes and that this NHE isoform can function to absorb fluid from the lumens of isolated rat and mouse cholangiocyte preparations.

bile secretion; bile duct epithelium; hepatocyte

Bile is a secretory product of both hepatocytes and cholangiocytes. Cholangiocytes line the biliary epithelium and are capable of both secretory and absorptive functions that modify the primary secretion from the liver parenchymal cells (6, 31). Although the mechanisms that determine the secretion of bile from hepatocytes have been largely characterized at the molecular level in recent years, less is known about the function of cholangiocytes, which represent only 3–5% of the total liver cell population. Long thought to be little more than a conduit for the delivery of primary bile to the intestine, this epithelium is capable of carrying out a variety of both secretory and absorptive processes, as indicated by more recent studies. Hormones such as secretin, vasoactive intestinal polypeptide (VIP), and bombesin have been shown to be direct stimulants of HCO3− secretion after meal-stimulated release of these hormones (1, 11, 13, 19). This process occurs through the activation of a cystic fibrosis transmembrane conductance regulator (CFTR) Cl− channel coupled with a Cl−/HCO3− exchanger on the apical domain (1, 19). Absorptive processes have been less well characterized, but transporters that mediate the removal of glucose (Glut-3) and bile salt (Isbt) have been described (16, 17). Purinergic receptors on the apical domain stimulate calcium-mediated Cl− secretion (20, 28). Less is known about alternative mechanisms for the transport of fluid and electrolytes, particularly whether NaCl and water are absorbed by the biliary epithelium and whether this process has a role in modulation of basal as well as hormone-stimulated ductular secretion.

In addition to a role in acid-base balance, Na+/H+ exchangers (NHE) play an important role in the absorption of NaCl in a number of epithelia (26). These transporters exist as a family of multiple isoforms with different tissue and regional distributions. At least five isoforms have been described. Recently, NHE3 has been localized to the apical domains of several epithelia, including the kidney (4, 9, 25), intestine (9), and submandibular gland (18), where it facilitates NaCl absorption. In the present study, we have identified NHE3 protein on the apical membranes of cholangiocytes and, for the first time, demonstrated a functional role for NHE3 in the absorption of fluid from the lumen.
of isolated bile duct units (IBDU) from the mouse. NHE3 was also detected in canalicular membranes of hepatocytes. These findings have important implications for understanding the regulation of salt and water secretion in cholangiocytes, both for the normal physiology of the biliary tree as well as in pathological states such as cholestatic liver disease.

MATERIALS AND METHODS

Materials. DNase, hyaluronidase, BSA, forskolin, penicillin-streptomycin, dexamethasone, insulin, and nigericin were purchased from Sigma Chemicals (St. Louis, MO). MEM, α-MEM, Williams’ E medium, Liebowitz 15 (L-15), and gentamicin were from Gibco (Grand Island, NY). FCS was from Gemini Bioproducts. Collagenase B was from Boehringer Mannheim, and 5-(N-ethyl-N-isopropyl)-amiloride (EIPA) was from RBI (Natick, MA). Pronase was from Calbiochem (La Jolla, CA), and Matrigel was purchased from Collaborative Biomedical.

Primary antibodies. Isoform-specific monoclonal antibodies (MAbs) and polyclonal (raised in guinea pig or goat) anti-B1 antibodies were raised to a maltose-binding protein fusion protein representing amino acids 702–831 of rabbit NHE3 (5, 27). The epitope for MAb 2B9 has been mapped to a region between amino acids 702 and 756, whereas the regions for MAbs 4F5 and 19F5 lie between amino acids 756 and 792. Anti-NHE3 MAbs 2B9, 4F5, and 19F5 were obtained from Chemicon International (Temecula, CA).

MAbs were used either as undiluted hybridoma supernatant or as purified IgG (stock solutions at ~1 mg/ml) at 1:50 for immunocytochemistry or at 1:10,000 for immunoblotting. Rabbit antisera was used at 1:50 for immunocytochemistry or at 1:1,000 for immunoblotting.

Secondary antibodies. For indirect immunofluorescence microscopy, Alexa 488 goat anti-mouse IgG and Alexa 594 goat anti-rabbit IgG (Molecular Probes) were used. For immunoblotting, horseradish peroxidase (HRP)-conjugated rabbit anti-goat IgG (heavy and light chain specific) was purchased from Zymed Laboratories (San Francisco, CA) and goat anti-rabbit and sheep anti-mouse peroxidase-conjugated F(ab’/2) IgG was from Sigma. For immunoelectron microscopy an HRP-conjugated goat anti-mouse (heavy and light chain specific) F(ab’/2) (Zymed Laboratories) was also used.

Cell isolation and culture. Male Sprague-Dawley rats (150–250 g) were purchased from Camm Laboratory Animals (Wayne, NJ). Female (+/+) and (−/−) NHE3 mice were established as previously described (29). Isolated rat hepatocyte couples (IRHC), cholangiocytes, and IBDU from rats and mice were isolated as previously described (8, 12, 14, 23). Briefly, the livers were perfused via the portal vein with Hanks’ A buffer for 10 min at 40 ml/min for rats and 5 ml/min for mice. This was followed by Hanks’ B buffer supplemented with 0.03% collagenase B for 10 min or until livers appeared digested. The livers were excised, and the hepatocytes were removed by gently shaking in 4°C L-15; the remaining nonparenchymal tissue was used for IBDU isolation. The hepatocytes were passed through 80- and 40-μm mesh to remove large clusters of cells and then washed three times in L-15 by centrifugation. Hepatocytes were plated on Matrigel-coated coverslip fragments in Williams’ E medium supplemented with 26 mM HCO3−, penicillin-streptomycin (100,000 units, 100 mg/l), 50 μg/ml gentamicin, 0.1 μM insulin, 0.3 μM dexamethasone, and 10% FCS and maintained in culture in a 5% CO2 air-balanced incubator at 37°C before use.

Immunocytochemistry. Rat livers were cut into 5-mm cubes and quick frozen in liquid N2-cooled Freon before sectioning. Cryosections were fixed in −20°C acetone for 10 min, washed in 0.1 M PBS, and blocked in 1% BSA for 30 min followed by 1-h incubation in anti-NHE3 (2B9) primary antibody diluted 1:50. After washing in PBS, an anti-mouse NHE3 secondary antibody (1:500) was added for 1 h and the sections were washed and mounted in Vectashield (Vector Laboratories, Burlingame, CA). Fluorescence was visualized on a Zeiss LSM 510 confocal microscope or a Nikon Microphot FX epifluorescence microscope.

Tissue preparation for electron microscopy. Sprague-Dawley rats were starved overnight but were allowed free access to drinking water. The animals were anesthetized with pentobarbital sodium and perfusion fixed via the left ventricle. Perfusion was performed first with PBS, pH 7.4, at 37°C to remove blood, followed by PLP fixative containing 2% paraformaldehyde, 75 mM lysine, and 10 mM sodium periodate in phosphate buffer, pH 7.4 (21). Blocks of tissue (2- to 4-mm cubes) from fixed livers were cut and postfixed in PLP for an additional 4–6 h at room temperature. Tissue was then washed in PBS and stored at 4°C in PBS containing 0.5% paraformaldehyde. Blocks of fixed tissue were incubated overnight in 30% sucrose in PBS, then frozen in liquid N2-cooled isopentane.

Immunoelectron microscopy. Immunoelectron microscopy was performed using the HRP method as described previously (3). Briefly, 300-μm cryosections of fixed liver were incubated in primary antibody overnight at 4°C in buffer containing 1% BSA in PBS (pH 7.4). The next morning, sections were washed in PBS and incubated in HRP-conjugated secondary antibody in the same buffer for 2 h at room temperature. After washing, the sections were fixed in 3% glutaraldehyde, reacted with diaminobenzidine, postfixed in OsO4, and embedded in Epon. Sections were cut, stained with lead citrate, and examined with a Zeiss 910 electron microscope.

Preparation of membrane fractions. Sprague-Dawley rats (Charles River) were killed by injection of pentobarbital sodium (Butler, Columbus OH). Brush border membrane vesicles (BBMV) were prepared from renal cortex using the Mg2+ precipitation method described previously (2).

Canalicular membranes from hepatocytes were isolated, as previously described by this laboratory (22). BBMV and canalicular membranes were used for SDS-PAGE and Western blotting.

SDS-PAGE and Western blotting. Protein for SDS-PAGE from hepatocytes and IBDU was isolated by washing away culture media with ice-cold Tris-sucrose (0.1 M Tris, 0.25 M sucrose) and then homogenizing by repeatedly passing through a 30-g needle in Tris-sucrose containing the protease inhibitors 0.2 mM phenylmethyisulfonfyl fluoride, 0.5 mM benzamidine, 5 μM leupeptin, and 1 μM aprotinin. The suspension was centrifuged at 1,000 g for 8 min, and the supernatant was assayed to quantitate protein. Samples were run on a 7.5% polyacrylamide gel and transferred to nitrocellulose paper or polyvinylidene
difluoride (PVDF) membranes (Millipore Immobilon-P). Immuno- 
noblotting was performed as follows. Sheets of nitrocellulose or 
PVDF containing transferred protein from gels were incubated 
first in Blotto (5% nonfat dry milk in PBS or Tris-buffered 
 saline, pH 7.4) for 1–3 h to block nonspecific binding of antibody 
and incubated with primary antibody (anti-B1 or MAb 2B9) 
diluted 1:500 overnight at 4°C. They were next washed in Blotto 
for 3 h, incubated in anti-goat (B1) or anti-rabbit (2B9) peroxi-
dase-conjugated IgG secondary antibody 1:2,000 for 1 h, washed 
again three times in Blotto and once in PBS or Tris-buffered 
saline, and detected using the ECL chemiluminescence system 
(Amersham, Arlington Heights, IL) according to manufacturer’s 
protocols.

Vi**deo**microscopy. IRHC and IBDU were cultured on cover-
slip fragments for 24 and 48 h, respectively, before being 
transferred to the stage of an inverted microscope (IM35 Zeiss, 
Thornwood, NY) equipped with Nomarski optics, where they 
were perfused at 37°C with Krebs-Ringer-bicarbonate buffer 
gassed with 95% O2-5% CO2. IRHC and IBDU were imaged 
with a charge-coupled device camera (Dage-MTI, Michigan 
City, IN) connected to a computer, where they were measured 
using image analysis software. The maximal cross-sectional 
area was measured and converted to volume using the formula 
$V = \frac{4}{3} \pi r^3$, where $r$ (radius) was derived from the maximal areas 
(MA): $MA = \pi r^2$ or $r = \sqrt{MA/\pi}$. This calculation assumes 
that the luminal area is spherical as judged from previous cross-
sectional analyses of the IBDU lumens (10). Data was stan-
dardized by expressing the changes in volume as percentage of 
baseline (time 0) values. Changes in luminal volume were 
assessed as a measure of net secretion (+) or absorption (−) by 
the cholangiocyte epithelium. Both IBDU and IRHC were stim-
ulated to secrete with 10 μM forskolin, which was infused as 
specified in each experiment. In experiments in which the NHE 
inhibitor EIPA (1 μM) was used, the coverslips were preincu-
bated for 30 min before the start of the experiment and infused 
throughout the experiment.

Statistical analysis was performed using unpaired Student’s $t$-test. Data are expressed as means ± SE.

**RESULTS**

**Immunolocalization of Na$^+$/$H^+$ exchanger isoform NHE3 in rat liver.** To localize Na$^+$/$H^+$ exchanger isoform NHE3 in the rat liver we used isoform-specific monoclonal antibody 2B9 combined with indirect immunoﬂuorescence microscopy. Liver sections showed strong immunoreactivity to NHE3 (Fig. 1). In hepatocytes, strong positive staining was limited to the canalicular membrane and no basolateral staining was 
observed (Fig. 1A). Staining was similar throughout the 
lobule and was not restricted to any particular zone. 
Bile ducts, regardless of their size, also stained 
intensely at their apical membrane (Fig. 1B). Sinusoid-
al endothelial cells demonstrated weak immunoreac-
tivity to the NHE3 antibody (Fig. 1A). Furthermore, 
expression of NHE3 was maintained in cultured hepatoc-
tocyte couplets (Fig. 1C), where it was also observed at 
the canalculus. The specificity of staining for NHE3 in 
rat liver was verified by the observation (not shown) 
that identical staining resulted from use of MAbs 4F5 
and 19F5, which react with a different epitope on 
NHE3 than does MAb 2B9 (5).

The expression of NHE3 on membranes of hepato-
cytes and cholangiocytes was further conﬁrmed by im-
muoelectron microscopy (Fig. 2). Figure 2A demon-
strates strong staining for NHE3 on the apical membrane domain of cholangiocytes. There was strong 
staining for NHE3 that was limited to the canalicular membrane of hepatocytes (Fig. 2B).

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Fig. 1. Immunofluorescence of Na$^+$/$H^+$ exchanger isoform NHE3 in rat liver. A: the distribution of NHE3 is restricted to the canalicular membrane in hepatocytes (arrows) and less intensely on sinusoidal endothelial cells (arrowheads). B: cholangiocytes in large and small bile ducts stain exclusively at the apical membrane (arrows). C: immunolabeling of NHE3 in cultured hepatocyte couplets, where staining is also highlighted at the apical canalicular membrane (arrow). Bar = 25 μm.
Immunoblotting. Western blot analysis of protein extracted from cultured IBDU revealed a positive band that migrated to the same position as protein from the kidney brush border positive control (Fig. 3A). The molecular weight was ~83–85, consistent with previous reports for NHE3 (4, 5). Although protein extracted from hepatocytes failed to demonstrate a definitive positive band regardless of whether the cells were freshly isolated or cultured for 24 h, detection of a positive band in purified canalicular membranes (Fig. 2).

Fig. 2. Immunoelectron microscopy: localization of NHE3 in cholangiocytes (A) and hepatocytes (B) in rat liver. PLP-fixed rat kidney was immunolabeled with anti-NHE3 MAb 4F5 using the immunoperoxidase method. In A, staining in cholangiocytes is restricted to the apical plasma membrane (large arrows). In B, staining in hepatocytes is seen on the canalicular membrane (large arrows). No staining is seen on the basolateral membrane (small arrows) or in any intracellular organelles. L, lumen; N, nucleus. Magnification: A = ×25,000, B = ×30,000.
NHE3 and Cholangiocyte Fluid Secretion and Absorption

skolin stimulated expansion of the luminal space by $126 \pm 38\%$ ($n = 10$) (Fig. 5A). Luminal expansion progressively diminished after withdrawal of forskolin to $1 \pm 23\%$ above baseline values. EIPA slowed forskolin’s maximum secretory response ($+80 \pm 21\%$, $n = 12$). However, in contrast to the control experiment, EIPA reduced the decline in luminal expansion after forskolin to $61 \pm 16\%$ by 45 min, consistent with involvement of an NHE in fluid reabsorption from the apical surface of NHE3 (+/+). A similar pattern was observed in rat IBDU (Fig. 5B). On stimulation with 10 μM forskolin, rat IBDU expanded by $137 \pm 29\%$ ($n = 15$), followed by a progressive decrease to $48 \pm 21\%$ above baseline values by the end of the experiment. Normal rat IBDU incubated in 1 μM EIPA maximally expanded by 103 ± 29%, and their luminal expansion only decreased to $88 \pm 22\%$ above baseline values by 50 min ($n = 12$).

To assess whether hepatocytes were also capable of fluid absorption from the canalicular lumen, wild-type mouse hepatocytes were incubated with forskolin (10 μM) for 8 min. The canalicular luminal area increased by $30 \pm 8\%$ at 10 min ($n = 9$; Fig. 6A). However, in contrast to IBDU, the lumen of the hepatocyte couplets continued to expand for another 10 min after withdrawal of forskolin to $44 \pm 9\%$ above baseline. These findings were also significantly different from the luminal expansion of control hepatocytes (+10 ± 10% at 20 min; $P < 0.05$) that received no forskolin.

Fig. 3. Immunoblot of NHE3 in bile duct units, hepatocytes, and canalicular membranes. A: immunoblotting with anti-NHE3 illustrates a strong band at ~80 kDa in kidney brush border membranes. Bile duct units, but not hepatocyte protein extracts, demonstrate a band at the same molecular mass as the control kidney membranes. B: proteins from rat canalicular membranes (left lane) or rat renal brush-border membrane vesicles (right lane) were separated by SDS-PAGE and prepared for immunoblotting. The blot was probed with anti-NHE3 MAb 2B9. Monomeric NHE3 appears as an 80- to 62-kDa band (arrow). Molecular weights, expressed as $10^3 M_r$, are presented on the left.

3B) confirmed that NHE3 protein is present in hepatocytes.

Functional assessment of NHE3. To determine a functional role for NHE3 we analyzed hepatocyte couplets and IBDU from normal and NHE3 (−/−) mice by videomicroscopy using techniques previously described in detail by this laboratory (7, 11, 23, 32). As illustrated in Fig. 4, when IBDU isolated from the wild-type NHE3 mouse (+/+) were perfused with forskolin (10 μM) for 10 min, the duct lumen rapidly expanded in response to this secretagogue, reaching a maximum expansion of $+87 \pm 20\%$ (relative to baseline, $n = 13$) 5 min after forskolin was withdrawn. Thereafter, the lumen volume progressively declined, returning to baseline ($−5 \pm 10\%$) by 30 min, consistent with either absorption or leakage of fluids from the closed luminal space. However, in the NHE3 (−/−) IBDU, the luminal volume did not decline after reaching maximum expansion during the 10-min forskolin infusion compared with controls ($+69 \pm 14\%$, $n = 22$) but remained expanded compared with controls ($68 \pm 21\%$ above baseline) for the duration of the experiment. This strongly suggests that NHE3 and not leakage is responsible for the observed fluid absorption from the cholangiocyte lumen. The role of cholangiocyte NHE3 in facilitating fluid absorption was examined further in rat and wild-type mouse IBDU by assessing the effect of the Na+/H+ exchange inhibitor EIPA (1 μM). In control experiments in NHE3 (+/+) mouse IBDU, for-
canaliculi maintained their luminal volume (+24 ± 6% in forskolin-stimulated vs. −8 ± 3% in controls at 20 min; P < 0.0001). These data suggest that fluid is not absorbed from the bile canaliculus of hepatocytes over this period of observation.

**DISCUSSION**

In the present study, we identified NHE3 on the luminal membranes of cholangiocytes and hepatocytes and demonstrated a role for this NHE isoform in fluid secretion and absorption from the luminal space of isolated mice and rat cholangiocyte preparations. NHE3 was localized by immunofluorescence staining to the apical membrane in both rat cholangiocytes and hepatocytes using isoform-specific MAb's. These sites of membrane expression of NHE3 were also demonstrated by immunoelectron microscopy. Western blot analysis confirmed the presence of NHE3 protein in cholangiocytes and in purified canalicular membranes, consistent with the findings of immunocytochemistry. We next used mice in which the NHE3 gene had been disrupted by gene targeting (29) to determine whether the NHE3 isoform was involved in absorption of fluid from the lumen of isolated cholangiocyte preparations. Net fluid secretion/absorption was assessed using IBDU from NHE3 (+/+ ) and (−/−) mice and videomicroscopic techniques. IBDU from rat and mice have been characterized extensively and are useful in vitro models to assess secretory responses in this epithelium. IBDU form closed spaces in short term culture and accumulate fluid within the spaces after exposure to various secretagogues including secretin, dibutyryl

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**Fig. 5.** A: luminal volume expansion was compared in wild-type mouse IBDU with (●, n = 12) and without (○, n = 10) infusion of 1 μM EIPA. After 10 μM forskolin, luminal volume returned to baseline in controls but stayed elevated in IBDU infused with EIPA. *P < 0.05. B: the same experiment was repeated in rat IBDU with similar results. Control IBDU ( ●) decreased toward baseline after removal of forskolin, whereas the luminal volume of IBDU incubated in EIPA showed minimal reductions (●).

**Fig. 6.** A: mouse hepatocyte couplets were used to assess fluid reabsorption from the canalicular lumen. After infusion of 10 μM forskolin between 2 and 10 min, there was a sustained canalicular secretory response (●, n = 9) compared with unstimulated hepatocytes (○, n = 8). *P < 0.05. B: the same experiment was performed with rat hepatocyte couplets. Canalicular area was virtually unchanged for 10 min after withdrawal of forskolin (●, n = 20) compared with unstimulated controls (○, n = 19). *P < 0.001.
cAMP, forskolin, VIP, and bombesin (1, 13, 23, 32). Measurements of cross-sectional diameters of this space by videomicroscopy have been validated as quantitative determinations of changes in volume within the duct lumens (32).

Using this approach, we have demonstrated that the NHE3 isoform is responsible for fluid absorption in the BDU, because fluid was not absorbed if NHE3 was inactivated by gene knockout or by the NHE inhibitor EIPA. This finding contrasts markedly with observations in the wild-type mouse and rat, in which the expanded luminal spaces of these BDU progressively diminish immediately after cessation of the forskolin infusion. The studies with EIPA further support a role for NHE isoforms in fluid absorption because a qualitatively similar pattern of inhibition of fluid absorption was observed after its administration. However, because EIPA would be expected to inhibit all NHE isoforms at this dose, we cannot exclude an indirect effect of other NHE isoforms on the luminal expansion in those inhibitor experiments. Also, because stimulation of luminal expansion during forskolin administration was slightly less in both the knockout and EIPA experiments, rather than enhanced above controls as might have been expected if absorption was inhibited, other nonspecific effects on the degree of luminal expansion cannot be excluded in these experiments.

Previous studies using isolated, perfused segments of rat bile ducts have also provided evidence for a functional NHE in the apical membrane that is distinct from a basolateral NHE because it is activated only at pH values <7.0 (30). NHE3 has also been detected in the apical membranes of several other polarized epithelial structures including the kidney, intestine, gallbladder, and salivary glands (4, 9, 18, 25). Limited functional studies have suggested that NHE3 is primarily involved in NaCl and NaHCO₃ absorption from these epithelia rather than pH regulation. The functional analyses in the present study are consistent with this property and provide the first direct demonstration suggesting that 1) fluid secretion is counterbalanced by fluid absorption in cholangiocytes in the normal resting bile duct epithelia and 2) inhibition of the process of absorption may contribute to net secretion after the stimulation of adenyl cyclase by forskolin. These findings add a new level of complexity to our understanding of the mechanisms of regulation of fluid secretion by the cholangiocyte epithelium.

Heretofore, fluid secretion from cholangiocytes has been thought to be regulated by cAMP-mediated activation of Cl⁻ secretion via a CFTR homologue in the apical membrane of cholangiocytes. This Cl⁻ channel is coupled to an apical Cl⁻/HCO₃⁻ exchanger on the apical domain, resulting in net secretion of HCO₃⁻ in the bile duct lumen (1, 19, 31). On the basis of the current studies and observations from other epithelia, a cAMP- and protein kinase A-mediated inhibition of NHE3 now also seems to be involved. How this effect of cAMP is mediated is not clear. However, studies in fibroblast cell lines suggest that for this phosphorylation to occur, additional regulatory proteins are required, known as NHE3 regulatory factor or NHE3 kinase A regulatory protein, that bind to NHE3 via PDZ domains (33). Recycling of NHE3 between subapical compartments and the apical domain of cholangiocytes might also be involved in the regulation of NHE3 activity, as described in other tissues (15). Further studies will be necessary to evaluate whether these mechanisms have a similar regulatory role for NHE3 in fluid secretion by cholangiocytes.

The finding of a NHE3 protein by immunofluorescence, immunoelectron microscopy, and Western blotting in the hepatocyte apical canalicular membrane of rat liver was unexpected. Previous functional studies in isolated basolateral and canalicular membrane vesicles from rat liver had found NHE activity only in basolateral membranes and not at the apical canalicular domain (24). Therefore, in the present study, we assessed this possibility further using isolated hepatocyte couplets from mouse and rat liver. This well-characterized model, like BDU, is a polarized secretory unit that can be maintained in short-term culture and permits assessment of fluid secretion from the bile canaliculus of the hepatocyte by videomicroscopy (7). As shown in this study, NHE3 was also detected by immunofluorescence microscopy on the apical canalicular domain of hepatocyte couplets (Fig. 1C). However, unlike BDU from normal rats and mice, normal mouse and rat hepatocyte couplets did not demonstrate a decline in luminal expansion immediately after cessation of the forskolin-induced secretory response, which would have been expected if absorption of fluid in the canalicular space were significant. Although we cannot exclude the possibility of fluid absorption over longer time periods, more prolonged observation of couplets is technically difficult because of cytoskeleton-mediated contractions that result in leakage from the closed canalicular space in this model. Thus both our previous studies in membrane vesicles (24) and the present findings suggest that NHE3 is not functional at the apical canalicular domain. Future studies will need to determine what factors may be responsible. In summary, the present study provides the first evidence for the presence of NHE3 in cholangiocytes and hepatocytes and demonstrates a functional role for NHE3 in fluid absorption from cholangiocyte epithelia. Further studies of the regulation of biliary fluid production must take into account the role of apical NHE3 in the net secretion of bile.

REFERENCES


