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Characterization of Volume-Activated Chloride Currents in Regulatory Volume Decrease of Human Cholangiocyte

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Abstract Volume-activated chloride channel (VACC) plays vital roles in many physiological functions. In bile duct epithelium, VACC actively participates in biliary secretion and cell volume regulation, and it mediates regulatory volume decrease (RVD). Recently, we have shown that mouse cholangiocytes have an intact RVD via VACC and K^+ conductance. However, such cell volume regulation was not studied in the normal human cholangiocyte. Volume measurement by Coulter counter and whole-cell patch clamp technique were used to characterize the RVD and VACC in human cholangiocyte cell line (HBDC). When exposed to hypotonic solution, HBDC exhibited an intact RVD, which was inhibited by 1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl ester) (BAPTA-AM), NPPB (5-nitro-2′- (3-phenylpropylamino)-benzoate), DIDS (4,4′-diisothiocyanatostilbene-2-disulfonic acid), and tamoxifen, but was not affected by the removal of extracellular calcium. During RVD, HBDC exhibited large, outwardly rectifying currents and time-dependent inactivation at positive potential. The amplitude of the outward current was approximately 3 times of that of the inward current, and this volume-activated current returned to the baseline when switched to isotonic solution. The amplitude and reversal potential of the volume-activated current was dependent on Cl^- concentration, and the VACC was significantly inhibited by replacing chloride with gluconate, glutamate, sucrose, and acetate in the hypotonic solution. In addition, classical VACC inhibitors, such as NPPB or tamoxifen, inhibited the VACC. These inhibitory effects were reversible with washing out the inhibitors from the bath solution. The present study is the first to characterize and show that HBDC has an intact RVD, mediated by VACC, which has similar electrophysiological characteristics as that in mouse cholangiocytes.

Keywords Human cholangiocyte · Cell volume · Volume-activated chloride channel · Regulatory volume decrease · Patch clamping

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Chloride channels play vital roles in maintaining ionic homeostasis in a variety of cell types by regulating cell volume and intracellular pH, as well as providing more specialized functions, such as transepithelial transport and regulation of excitability in the nerve and muscle tissues (Mintenig et al. 1993). The volume-activated chloride channel (VACC) mediates the regulatory volume decrease (RVD) against cell swelling, which involves the extrusion of intracellular K^+ and Cl^- ions when the cells are exposed to hypotonic solution (Hoffmann and Simonsen 1989; Kirk 1997; Mintenig et al. 1993). VACC are shown to play a central role in RVD, in conjunction with K^+ channels (Mintenig et al. 1993; Nilius and Droogmans 2003).

Previously, a number of different chloride channels have been identified in the bile duct epithelial cells or cholangiocytes using various cellular, electrophysiological, and radioisotope efflux studies in rat (Fitz et al. 1993), mouse

(Cho 2002; Cho et al. 2004), and human cholangiocytes (Roman et al. 1996). In addition, cell volume studies in human cholangiocarcinoma cell line (Roman et al. 1996) and mouse cholangiocyte cell line (Chen et al. 2004, 2007), and freshly isolated bile duct cell cluster (Cho 2002) have shown that cholangiocytes exhibit RVD when exposed to hypotonic solution by expelling intracellular K^+ and Cl^- ions via volume-activated K^+ channels and VACC, respectively (Cho 2002; Nilius et al. 1997).

The cholangiocarcinoma cell line and rodent cholangiocyte models are invaluable for studying cell volume regulation in the cholangiocytes. However, the results from those cell lines and models were questioned whether they could be applicable to human cholangiocyte physiology as a result of the malignant nature of the cholangiocarcinoma cell line and potential interspecies difference of rodent cholangiocyte models. Until recently, no well-characterized human cholangiocyte cell line was available to study human bile duct physiology, and no detailed and systematic study on RVD or electrophysiological characterization of VACC has been performed on normal human bile duct cells to examine their function and regulation in the human bile duct epithelium. Thus, the present study was undertaken to examine the ion transporters involved in the RVD using volume measurement and to perform detailed electrophysiological characterization of the chloride conductance pathways using whole-cell patch clamp techniques in bile duct cell line (HBDC), which is conditionally immortalized by SV40 virus and has previously been extensively characterized and used as a human cholangiocyte cell model (Bhaskar et al. 1998; Grubman et al. 1994, 1995). This work is the first in characterizing RVD and VACC in human cholangiocytes and should provide an important framework for further studies of VACC in human cholangiocytes.

Materials and Methods

Materials

Bovine serum albumin, penicillin/streptomycin, EGTA (ethylene glycol-bis (b-aminoethyl ether)-N,N′,N-tetraacetic acid), HEPES (4-(2-hydroxy-ethyl)-1-piperazine ethanesulfonic acid), D(+)-glucose, dimethyl sulfoxide, tamoxifen, sodium gluconate, L-thyroxine, prostaglandin E1, hydrocortisone, and epinephrine were purchased from Sigma (St. Louis, MO). Matrigel, dispase, epidermal growth factor, insulin, transferrin, and selenium were from Collaborative Biomedical (Bedford, MA). Dulbecco modified Eagle medium/nutrient mixture F12 Ham, fetal calf serum, and trypsin were from Gibco (Grand Island, NY). DIDS (4,4′-diisothiocyanatostilbene-2-disulfonic acid) was purchased from Molecular Probes (Eugene, OR) and NPPB (5-nitro-2′- (3-phenylpropylamino)-benzoate) from Calbiochem (San Diego, CA). The SV-40 transformed, conditionally immortalized human bile duct cell line was generously provided by Dr. Jefferson (Boston, MA).

Solutions

Isotonic and hypotonic bathing solution compositions were as described previously (Chen et al. 2004, 2007) and are briefly outlined in Table 1. The actual osmolarities of the solutions used were determined by Vapor Pressure Osmometer 5500 (Wescor, Logan, UT). Pipette solution (in mM) was as follows: CsCl 100, NaCl 10, $MgCl_2$ 1, $CaCl_2$ 0.1, HEPES 10, EGTA 1, Sucrose 60, MgATP 5, and Na_2GTP 0.1. Osmolarity was 285 mosM; pH was adjusted to 7.3 with CsOH.

Cell Culture

The HBDC is conditionally immortalized by SV40 virus and was shown to be stable, well-differentiated, and polarized cholangiocytes, which express cytokeratin 19, γ-glutamyl transpeptidase, and ion transporters (Bhaskar et al. 1998; Grubman et al. 1994, 1995), confirming its biliary cell origin and phenotype. These cells were grown in monolayer in the culture media as previously described (Bhaskar et al. 1998): Dulbecco modified Eagle medium/nutrient mixture F12 Ham (3:1) supplemented with the following: adenine (1.8×10^{-4} M), insulin (5 μg/ml), transferring (5 μg/ml), triiodothyronin (2×10^{-9} M), hydrocortisone (1.1×10^{-6} M), epinephrine (5.5×10^{-6} M), 10% fetal bovine serum, 100 mg/l penicillin G sodium, 100 mg/l streptomycin sulfate, and 10 ng/ml epidermal growth factor. The cells were grown on a flask at 37°C in a humidified atmosphere of 5% CO_2, and media were replaced every 2–3 days. The cells were passaged every 10–12 days after digestion with 0.05% trypsin.

Cell Volume Measurement

Mean cell diameter was measured in cell suspensions at 25°C by utilizing the Coulter Multisizer 3 and Multisizer software (version 3.51) (Beckman Coulter, Fullerton, CA) with an aperture tube diameter of 100 μm; mean cell volume (MCV) was calculated by the formula $4/3\pi r^3$. The instrument was calibrated with polystyrene beads of known dimensions (Beckman Coulter). Approximately 8 million cells grown on polystyrene flasks (Costar) were collected with 0.05% trypsin, suspended in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline and cell culture medium (to inactivate trypsin), centrifuged for 1 min at 1000 revolutions/min ($2000 \times g$), resuspended in 4 ml of isotonic

Table 1 Ionic compositions (in mM) of bathing solution

Solution	NaCl	Sucrose	KCl	$CaCl_2$	$MgCl_2$	KH_2PO_4	HEPES	Glucose	Osmolarity
Isotonic	100	80	4	1	2	1	10	5	304
Hypotonic	100	0	4	1	2	1	10	5	225
Ca-free hypo	100	0	4	EGTA 1	2	1	10	5	225
Hypotonic-gluconate	Na-Glu 100	0	4	1	2	1	10	5	221
Hypotonic-glutamate	Na-Gln 100	0	4	1	2	1	10	5	221
Hypotonic-acetate	Na-Acet 100	0	4	1	2	1	10	5	221
Hypotonic-sucrose		200	4	1	2	1	10	5	221

buffer, and incubated with gentle agitation for 45 min. Aliquots (500 µl) of isotonic cell suspension were then added to 100 ml of either isotonic or hypotonic buffers at time 0 min. Measurements were obtained from ~10,000 cells at −5 min (basal isotonic cell volume), and at every 1 min after exposure to control or experimental buffers. To chelate $[Ca^{2+}]_i$, the intracellular calcium chelator 1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl ester) (BAPTA-AM) (30 µM) was added to isotonic buffer during the 5 min of preincubation before hypotonic stress. Changes in size over time are expressed as relative volume percent changes by normalizing volume to the basal volume during the preincubation period, and control and experimental values are presented as percent ± standard deviation (SD) for statistical comparison of various conditions.

Patch Clamp

The patch clamp experiments were as described previously (Chen et al. 2004, 2007). Briefly, the cells were plated on matrigel coated rectangular glass coverslips (2–4 mm) and cultured at 37°C. After the cells were cultured 2–3 days, they were moved to a specimen chamber on an inverted Nikon microscope and perfused with bath solution at room temperature 23–25°C. Whole-cell currents were measured in the conventional configuration of the patch clamp technique with an Axopatch-1D amplifier (Axon Instruments, Foster City, CA). Pipettes were prepared (resistance 4–5 MΩ) from borosilicate capillary tubes (1.0 mm outer diameter, 0.5 mm inner diameter, Sutter Instruments, Novato, CA) with a P-87 Flaming/Brown micropipette puller (Sutter Instruments, San Rafael, CA). The junction potentials were determined by immersing the pipette into the bath filled with pipette solution, zeroing the voltage reading (Li et al. 2000). After forming a gigaseal, the fast compensation system of the amplifier was used to compensate for the intrinsic input capacitance of the head stage and the pipette. The membrane capacitance and series resistance were compensated (80% compensation) to minimize the capacitive transient and to improve the dynamic response. An Ag/AgCl electrode was used as the reference electrode. The current–voltage relationships between −120 and 130 mV were measured by voltage steps in 10 mV increments 200 ms, 1 Hz, and holding potential 0 mV in isotonic and hypotonic bathing solutions as indicated. The current was acquired and analyzed by pClamp 6.0 and pClamp 8.0 software (Axon Instruments, Foster City, CA), respectively. The current amplitude is measured at the end of the 200 ms pulse. For determining the relative permeabilities of various anions, 91% of the chloride in the hypotonic solution was replaced with other anions.

Data and Statistical Analysis

All measurement data are expressed as mean ± SD. Excel software (Microsoft, San Diego, CA) was used to perform Student's paired and unpaired *t*-tests for statistical data analysis. The E_{rev} (reversal potential) was estimated from linear regression analysis.

Results

Cell Volume

Under isotonic conditions, mean cell diameter was 14.21 ± 0.46 µm corresponding to MCV of 1435 ± 132.38 μm^3. As shown in Fig. 1a, exposure to hypotonic buffer caused a rapid initial increase, which correspond to a relative cell volume of 1.19 ± 0.036 ($n = 5$; $P < 0.001$). Such rapid swelling was followed by gradual recovery to a relative volume of 1.11 ± 0.01 by 10 min and 1.04 ± 0.03 at 30 min. An elimination of Ca^{2+} from hypotonic solution had no significant effect on RVD (Fig. 1b). However, exposure to hypotonic solution containing calcium chelator BAPTA-AM (30 µM), the RVD was significantly inhibited, and the relative MCV was 1.12 ± 0.04 at 30 min (Fig. 1c; $P < 0.001$) compared with control (Fig. 1c). As shown in Fig. 1d–f, coadministration of representative chloride channel inhibitors, NPPB, DIDS, or tamoxifen, with the

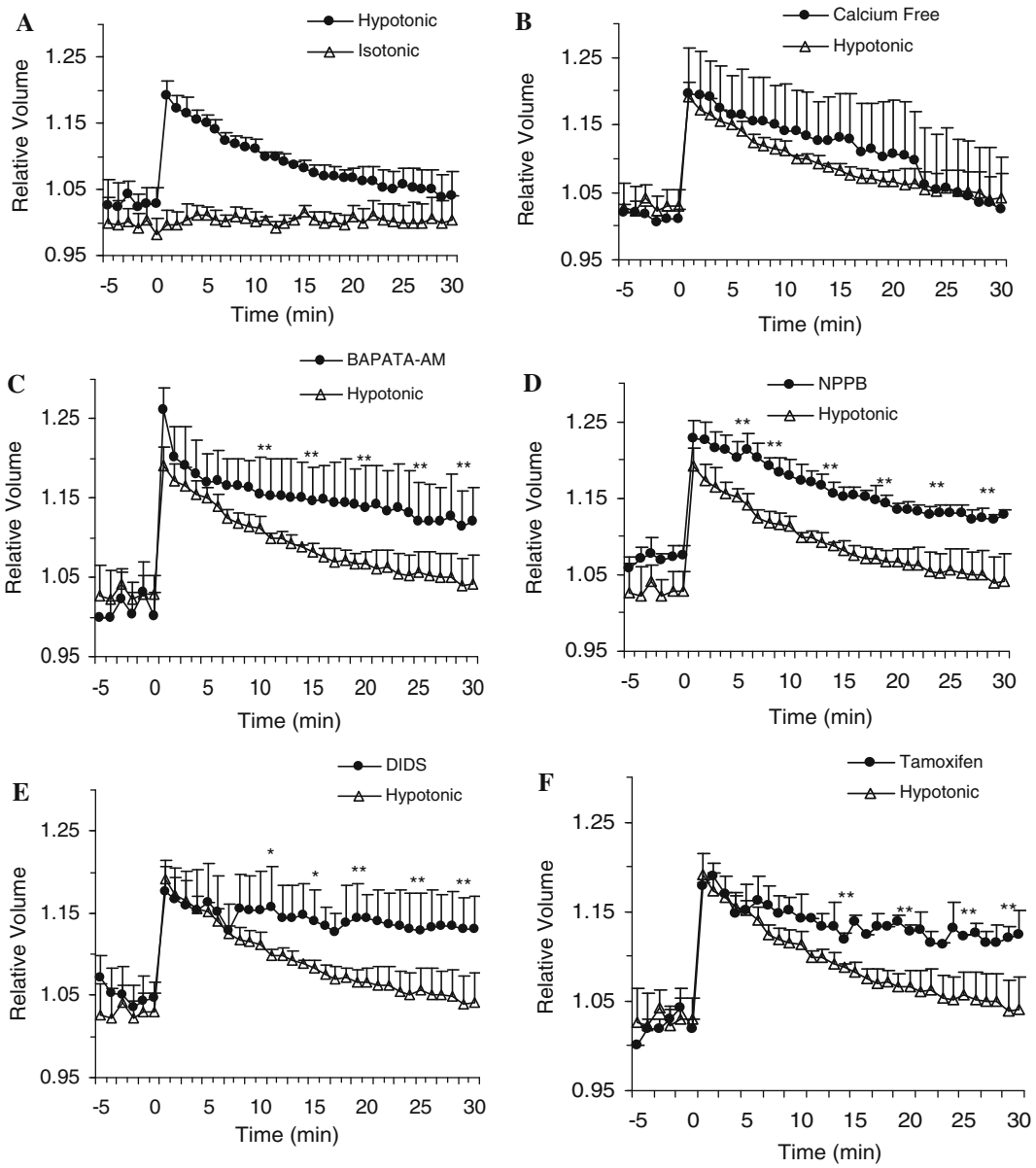


Fig. 1 Cell volume measurement and effect of Ca^{+2}, BAPTA-AM, NPPB, DIDS, and tamoxifen on RVD of human cholangiocyte cell line. **a** Control measurements during 30-min exposure to isotonic or hypotonic. Initial swelling in hypotonic solution was followed by RVD. **b** Calcium free extracellular solution. **c** BAPTA-AM 30 μM. **d** NPPB 40 μM. **e** DIDS 250 μM. **f** Tamoxifen 2 μM, $n = 5$–6, mean ± SD, * $P < 0.05$, ** $P < 0.01$

hypotonic solution inhibited the RVD in these cells, and the relative MCV after 30 min of hypotonic challenge was 1.07 ± 0.02, 1.09 ± 0.04 and 1.12 ± 0.04 which was statistically significant ($P < 0.05$) compared with the RVD (1.003 ± 0.02).

Membrane Current Recordings in Isotonic and Hypotonic Solution

To examine the effects of hypotonic challenge on the membrane currents, the basal currents were measured in isotonic solution; then the volume-activated currents were measured after switching the bathing solution to hypotonic solution. Figure 2 shows the recordings of typical membrane current and current–voltage relationship curves (a), time course (b), and summary (c). As shown in Fig. 2, the voltage step elicited baseline currents that were small, outwardly rectified current with a reversal potential of −0.23 ± 1.5 mV ($n = 4$, vs. calculated value of −1 mV). When the HBDC was exposed to hypotonic solution, significantly ($n = 6$, $P < 0.01$) greater currents (7.04 ± 1.80 nA in hypotonic solution vs. 0.23 ± 0.11 nA in isotonic solution on HBDC) at 120 mV were observed within 3–5 min and reached a peak within 5–10 min. These

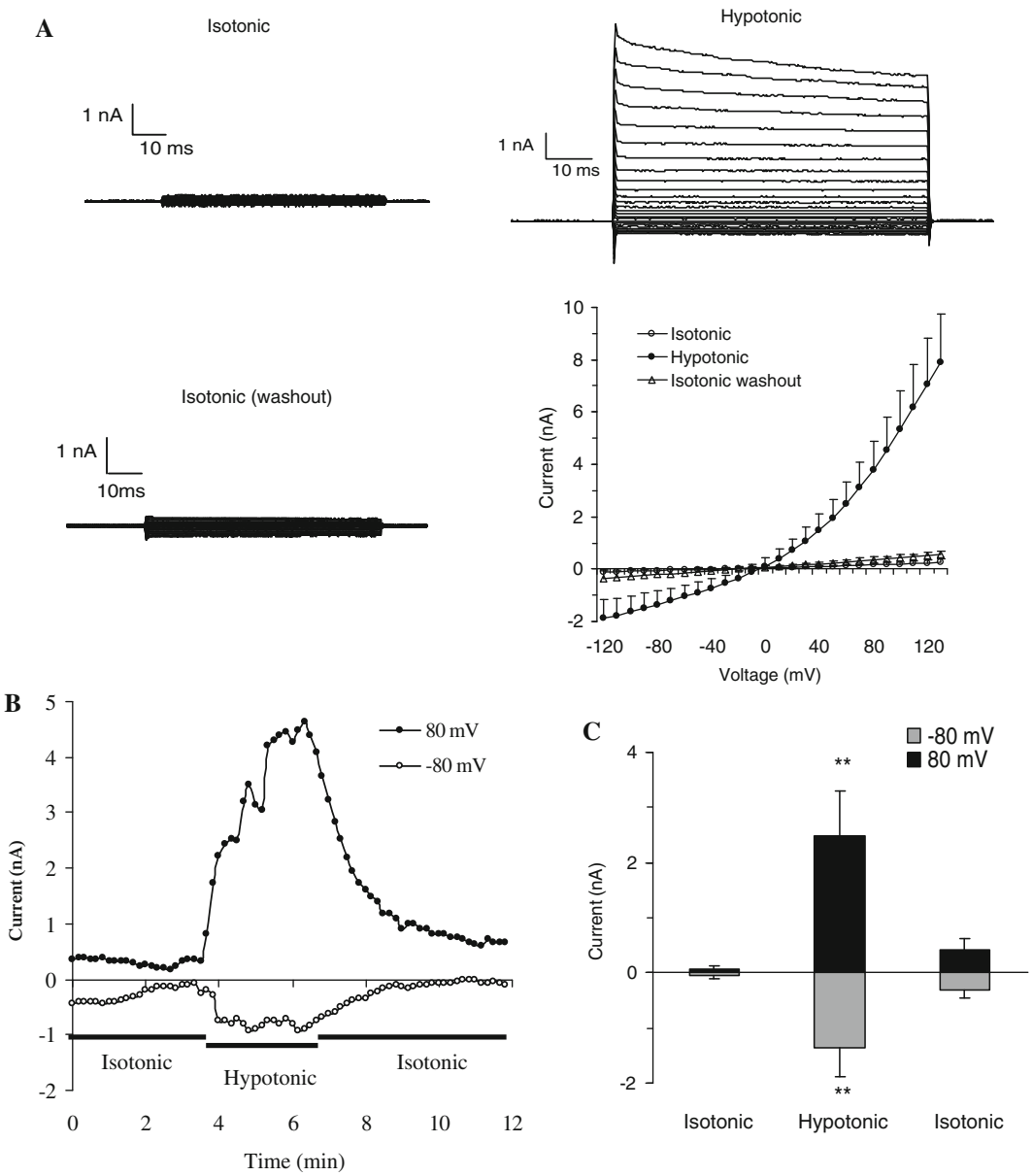


Fig. 2 Volume-activated chloride current in human cholangiocyte cell line. Whole-cell currents were measured using isotonic standard bathing solution or hypotonic solution and pipette solution. Currents measured using voltage steps from −120 to 130 mV in 10 mV increments, 200 ms, holding potential 0 mV. **a** Representative traces of chloride current and I–V curves. **b** Time course of current activated by hypotonic solution, 0 mV holding potential; 80 and −60 mV voltage steps for 200 ms. **c** Summary of currents activated by hypotonic solution in normal cholangiocyte cell line; $n = 6$, mean ± SD

currents showed outwardly rectified configuration with time-dependent inactivation like the basal currents. As shown in Fig. 2a, the amplitude of the initial currents recorded at +120 mV was approximately 3.9 times that of the currents recorded at −120 mV and their reversal potential did not shifted significantly (0.8 ± 1.9 mV, $n = 4$, $P > 0.05$ vs. calculated value of 1 mV) from baseline of 0 mV. When the cells were reexposed to isotonic solution, these volume-activated currents returned back to baseline level. The summary of these results are presented in Fig. 2c, which shows that hypotonic challenge significantly stimulated membrane currents and these volume-activated currents returned back to basal level when the cells were reexposed to isotonic solution.

Effects of Chloride Channel Blockers on Chloride Currents

To further characterize the volume-activated current, the effect of chloride channel blockers NPPB (40 μM) (Fig. 3a) and tamoxifen (2 μM) (Fig. 3b) on the volume-activated currents were studied. Figure 3 shows representative time

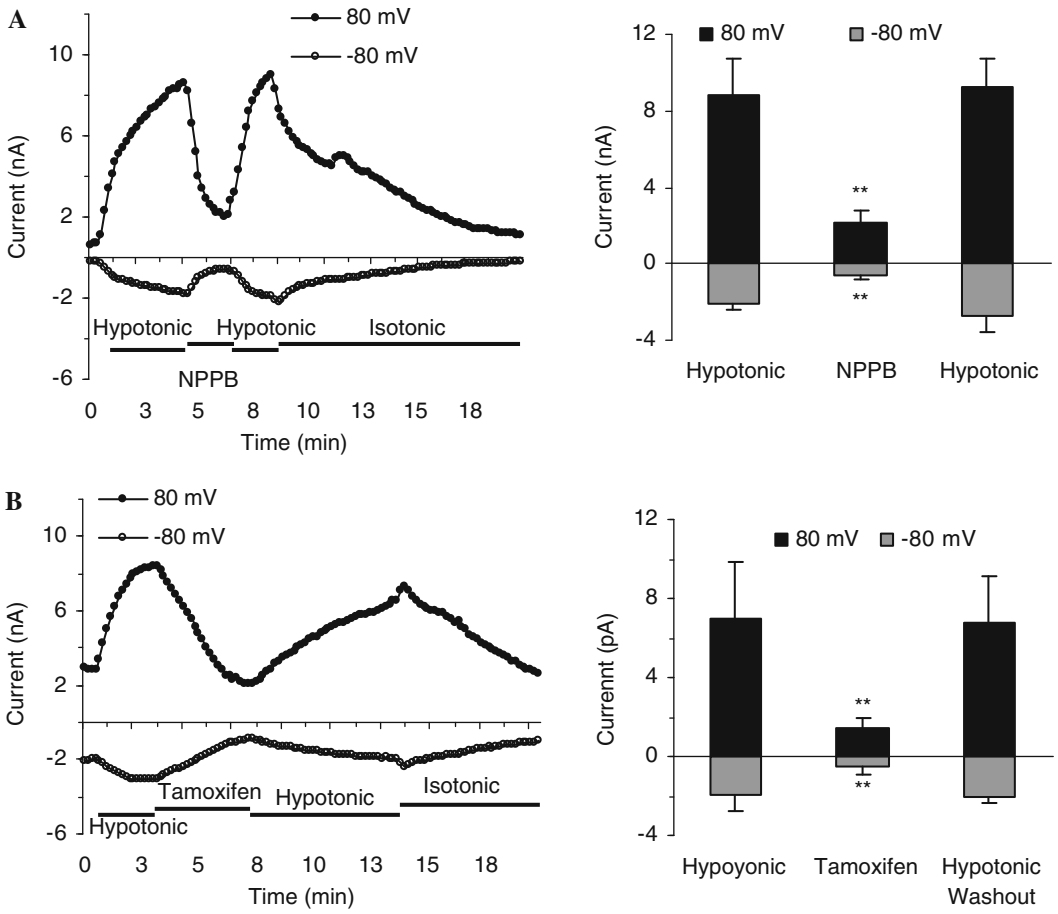


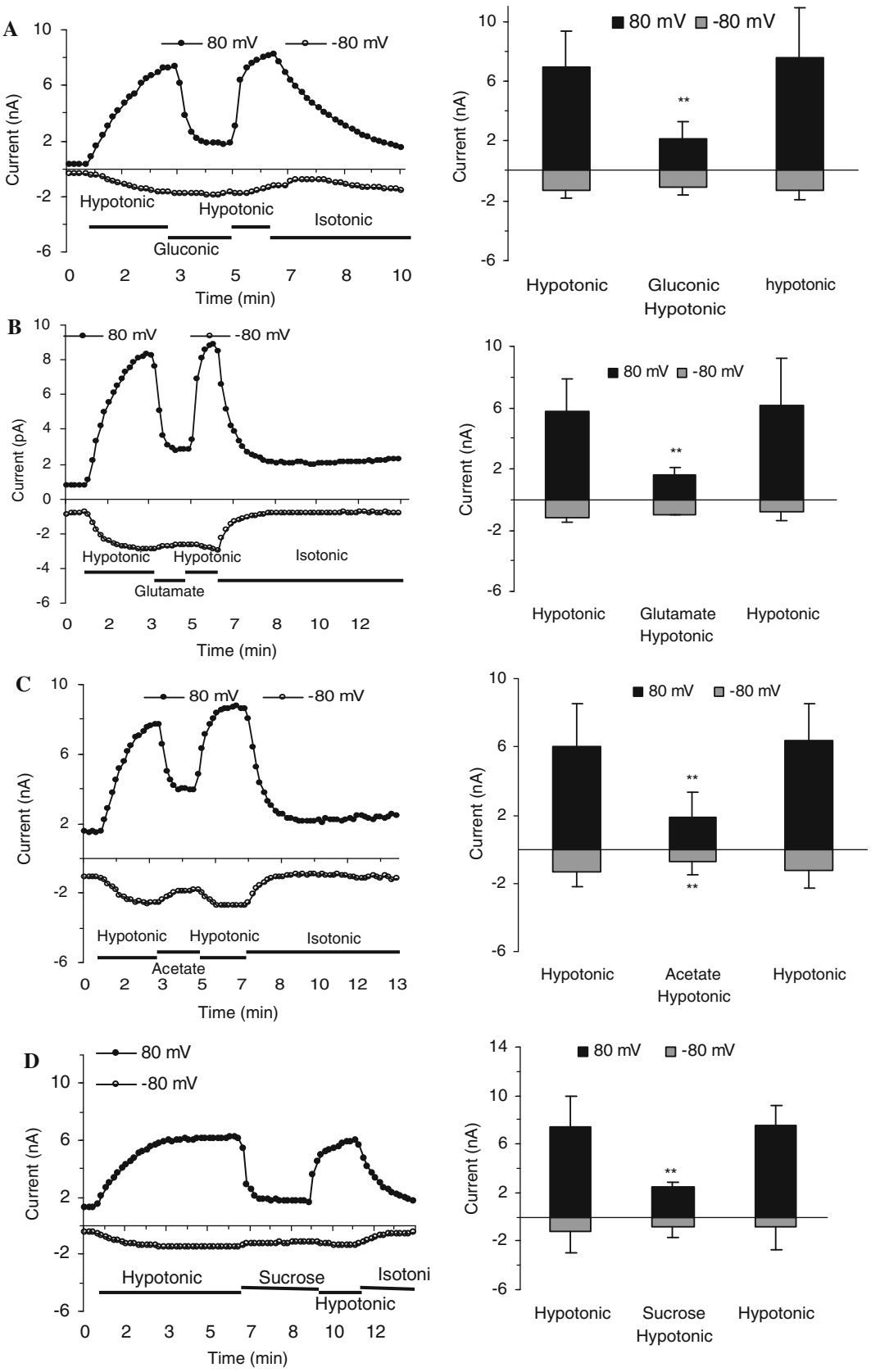
Fig. 3 Effects of NPPB and tamoxifen on chloride currents in human cholangiocyte cell line. The currents were measured by a voltage step 80 and −80 mV, 200 ms, 0.1 Hz, holding potential 0 mV. Representative time course and summary of effects of NPPB 40 μM (**a**) and tamoxifen 2 μM (**b**) on outwardly and inwardly chloride currents; mean ± SD, $n = 5$–6, ** $P < 0.01$

course and mean value of the effect of NPPB 40 μM (Fig. 3a) and tamoxifen 2 μM (Fig. 3b) on the outward and inward currents. The currents decreased significantly ($P < 0.01$ vs. control) when the cells were perfused with hypotonic solution containing NPPB (40 μM) or tamoxifen (2 μM), but the currents returned to high level when the cells were perfused with hypotonic solution without NPPB ($P < 0.01$ vs. NPPB, $P > 0.05$ vs. control) or tamoxifen ($P < 0.01$ vs. NPPB, $P > 0.05$ vs. control). These results indicate that the volume-activated current is significantly and reversibly inhibited by NPPB, a general chloride channel blocker, as well as by tamoxifen, a nonsteroidal antiestrogen derivative, known as one of the most potent and specific inhibitors of VACC (Mintenig et al. 1993; Schmid et al. 1998).

Effects of Replacing Extracellular Chloride with Gluconate, Glutamate, Acetate, and Sucrose on Volume-activated Current

To determine the role of chloride in the observed membrane currents during the hypotonic challenge, the effect of replacing extracellular chloride with gluconate, glutamate, acetate, and sucrose on inward and outward currents was examined. Figure 4 shows the time course of currents and summary of currents evoked by hypotonic chloride replacement with gluconate, glutamate, acetate, and sucrose. Under basal conditions in isotonic solution, the voltage step protocol elicited a small current, but in hypotonic solution, these voltage steps induced a large increase of whole-cell current ($P < 0.01$ vs. control isotonic). However, when the cells were exposed to the hypotonic solution with gluconate, acetate, glutamate, and sucrose replacing chloride, the currents were decreased significantly ($P < 0.01$ vs. hypotonic solution) by more than 70% ($Cl^- >$ acetate ≥ glutamate ≥ gluconate ≥ sucrose). The reversal potentials were 18.0 ± 10.0, 28.5 ± 11.2, 36.5 ± 17.0, and 25.0 ± 13.2 mV in the

Fig. 4 Effect of substitution of chloride with gluconate, glutamate, acetate, and sucrose on chloride currents in human cholangiocyte cell line. Representative time course and summaries of the effect of substitution of chloride with gluconate (**a**), glutamate (**b**), acetate (**c**), and sucrose (**d**) on volume activated chloride current, holding potential 0 mV, voltage step 80 and −80 mV, 100 ms; 0.1 Hz in normal human cholangiocyte cell lines; mean ± SD, $n = 5$–6, ** $P < 0.01$ ▶


A
80 mV
-80 mV
Current (nA)
Hypotonic
Hypotonic
Isotonic
Gluconic
Time (min)
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hypotonic
B
Current (pA)
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hypotonic solutions when chloride was replaced by gluconate, acetate, glutamate, and sucrose, respectively ($P > 0.05$ among all four groups). When the cells were reexposed to hypotonic solution containing chloride, the currents promptly returned to the previous high level. Again, when the cells were exposed to isotonic solution, the currents returned to the basal level ($P < 0.01$ vs. hypotonic solution, $P > 0.05$ vs. control isotonic solution) and the reversal potential also returned to near zero (0.35 ± 1.25 mV).

Discussion

The HBDC cell line used for the present study is phenotypically normal human cholangiocytes, which were conditionally immortalized by SV40 virus, and had the expected protein expression for bile duct–specific cytokeratin-19, confirming its biliary origin, and formed duct-like structures when grown in high density (Bhaskar et al. 1998; Grubman et al. 1994, 1995). Thus, considering the known limitations working with cholangiocytes as a result of difficulty in cell isolation and culture, this well-differentiated, polarized HBDC line should provide an invaluable cholangiocyte cell model to study human bile duct biology and physiology.

Previously, Mz-ChA-1 cholangiocarcinoma cell line (Roman et al. 1996) has been shown to have an intact RVD, which is dependent on both Cl^- and K^+ conductance as in other cell types (Banderali and Roy 1992; Hazama and Okada 1988; Mennone et al. 1995; Nilius et al. 1997) as well as in mouse cholangiocytes (Cho 2002). Likewise, our volume measurements in the present study show that HBDC also has an intact RVD, which is not affected by removal of extracellular calcium in the bath solution. However, as shown in Fig. 1c, chelation of intracellular calcium by BAPTA-AM has a significant inhibition on RVD as shown with mouse cholangiocytes (Chen et al. 2007). Our recent work has shown that intracellular calcium plays a permissive role in RVD in cholangiocytes (Park et al. 2007). Further characterizations of the RVD in HBDC using chloride channel blockers show that RVD is mediated by chloride channels that are inhibited by NPPB, DIDS, and tamoxifen, thus indicating the important role of VACC in RVD of human cholangiocytes.

Furthermore, our electrophysiological characterizations of HBDC using whole-cell patch clamping technique indicate that HBDC has VACC with similar electrophysiological characteristics as those reported in the literature (Chen et al. 2004; Mintenig et al. 1993; Nilius and Droogmans 2003; Roman et al. 1996) (Table 2). The VACC in many cell types have a number of typical electrophysiological characteristics (Mintenig et al. 1993). Their currents are low in amplitude in isotonic conditions but are activated by hypotonic challenge as shown in Fig. 2. In addition, the VACC currents show an outward rectification with a varying degree of time-dependent inactivation at depolarizing potentials depending on cell types (Chen et al. 2004; Lewis et al. 1993; Mintenig et al. 1993). Likewise, the volume-activated currents in HBDC show expected outwardly rectified currents and have a

Table 2 Comparison of volume-activated chloride currents in immortalized mouse cholangiocyte cell line, human cholangiocarcinoma cell line Mz-Cha-1, and immortalized human normal cholangiocyte cell line[a]

Characteristic	MBDC (4)	Mz-Cha-1 (4)	HBDC
Reversal potential			
Isotonic (mV)	0	0	−0.23
Hypotonic (mV)	2.2	2.4	0.8
Amplitude			
Isotonic			
100 mV (pA/pF)	16.51 ± 11.86	16.13 ± 17.11	14.75 ± 6.90
−100 mV (pA/pF)	12.37 ± 4.59	8.94 ± 7.45	6.85 ± 7.95
Hypotonic			
60 mV (pA/pF)	189.57 ± 53.87	195.42 ± 23.24	192.86 ± 62.29
−80 mV (pA/pF)	129.43 ± 66.76	122.29 ± 12.67	104.62 ± 40.82
Outwardly rectifying	Yes	Yes	Yes
Inactivation at high potential depolarization (> 60 mV)	Yes	Yes	Yes
Inhibitor (% of inhibition)			
NPPB (40 μM)	49%	63%	66%
Tamoxifen (2 μM)	73%	94%	80%

[a] Values for amplitude are mean ± SD. *MBDC* mouse bile duct cell, *HBDC* human bile duct cell, *NPPB* 5-nitro-2-(3-phenylpropylamino)-benzoate

time-dependent inactivation in high depolarized potential (>60 mV) as shown in Fig. 2. VACC has anion permeability selectivity of $Cl^- >$ acetate $\sim =$ glutamate $\sim =$ gluconate $\sim =$ sucrose) as shown in Fig. 4. These electrophysiological characterizations of the observed chloride channels are consistent with VACC. In addition, our present findings indicate that the results from the conditionally transformed HBDC is comparable to those from a human cholangiocarcinoma cell line and mouse cholangiocyte cell line (Chen et al. 2004; Roman et al. 1996). As expected, the VACC in HBDC and human cholangiocarcinoma cell and mouse cholangiocyte lines are activated with hypotonic challenge and exhibit outwardly rectified chloride current, which have a time-dependent inactivation at high depolarization voltages. The reversal potentials in isotonic solutions are 0 mV.

Moreover, the VACC was further characterized and confirmed as VACC by using a number of specific chloride channel inhibitors. The significant inhibition of the volume-activated currents observed with conventional chloride channel blockers such as NPPB and DIDS indicates that they are chloride channels (Mignen et al. 1999). In addition, the significant inhibition by tamoxifen, one of the most potent and specific inhibitors of VACC, provides a compelling evidence that the observed volume-activated current is, in fact, a VACC (Mintenig et al. 1993; Schmid et al. 1998). The observed inhibitory effects of these chloride channel inhibitors on VACCs were reversible after removing the inhibitors from the perfusion solution, indicating their specific inhibition, rather than nonspecific rundown of current.

As conclusion, to our knowledge, the present study is the first to show the important role of VACC in RVD in human cholangiocytes as well as provide the first detailed characterization of VACCs in human cholangiocytes involved in RVD. Although much of research has been performed in rodent cholangiocyte cell models in the past, our efforts to study human cholangiocytes have been limited as a result of scarce availability of normal and pathological human cholangiocyte cell models. Our results here show that HBDC contains an outwardly rectified VACC, which is activated by hypotonic challenge, has characteristic $\sim$0 mV reversal potential and is inhibited by chloride replacement in the perfusion solution and classical chloride channel inhibitors such as NPPB and tamoxifen. These results indicate that RVD and VACC in the human cholangiocytes are similar to those in mouse cholangiocytes and human cholangiocarcinoma cells (Table 2). In previous studies based on rodent and cholangiocarcinoma cells, RVD and VACC in cholangiocytes have been shown to play important roles in cell volume regulation, bile secretion, pH regulation, and so on, which are likely to also be true with human cholangiocytes, given the similar findings in this study. In conclusion, our study is novel in that it provides a detailed characterization of RVD and VACC in human cholangiocytes and should provide an important framework to further understand and study their role in biology and pathophysiology of human cholangiocytes in future studies.

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References

Banderali U, Roy G (1992) Activation of K^+ and Cl^- channels in MDCK cells during volume regulation in hypotonic media. J Membr Biol 126:219–234

Bhaskar KR, Turner BS, Grubman SA, Jefferson DM, LaMont JT (1998) Dysregulation of proteoglycan production by intrahepatic biliary epithelial cells bearing defective (Delta-f508) cystic fibrosis transmembrane conductance regulator. Hepatology 27:7–14

Chen BY, Nicol G, Cho WK (2004) Electrophysiological characterization of volume activated chloride currents in mouse cholangiocyte cell line. Am J Physiol Gastrointest Liver Physiol 287:1158–1167

Chen BY, Nicol G, Cho WK (2007) Role of calcium on volume activated chloride current in mouse cholangiocyte. J Membr Biol 215:1–13

Cho WK (2002) Characterization of regulatory volume decrease in freshly isolated mouse cholangiocytes. Am J Physiol Gastrointest Liver Physiol 283:G1320–G1327

Cho WK, Siegrist VJ, Zinzow W (2004) Impaired regulatory volume decrease (RVD) in freshly isolated cholangiocytes from cystic fibrosis mice. J Biol Chem 279:14610–14618

Fitz JG, Basavappa S, McGill J, Melhus O, Cohn JA (1993) Regulation of membrane chloride currents in rat bile duct epithelial cells. J Clin Invest 91:319–328

Grubman SA, Perrone RD, Lee DW, Murray SL, Rogers LC, Wolkoff LI, Mulberg AE, Cherington V, Jefferson DM (1994) Regulation of pHi by immortalized human intrahepatic biliary epithelial cell lines. Am J Physiol Gastrointest Liver Physiol 266:G1060–G1070

Grubman SA, Fang SL, Mulberg AE, Perrone RD, Rogers LC, Lee DW, Armentano D et al (1995) Correction of the cystic fibrosis defect by gene complementation in intrahepatic biliary epithelial cell lines derived from patients with cystic fibrosis. Gastroenterology 108:584–592

Hazama A, Okada Y (1988) Ca^{2+} sensitivity of volume-regulatory K^+ and Cl^- channels in cultured human epithelial cells. J Physiol 402:687–702

Hoffmann EK, Simonsen LO (1989) Membrane mechanisms in volume and pH regulation in vertebrate cells. Physiol Rev 69:315–382

Kirk K (1997) Swelling-activated organic osmolyte channels. J Membr Biol 158:1–16

Lewis RS, Ross PE, Cahalan MD (1993) Chloride channels activated by osmotic stress in T lymphocytes. J Gen Physiol 101:801–826

Li GR, Yang BF, Sun HY, Baumgarten CM (2000) Existence of a transient outward K current in guinea pig cardiac myocytes. Am J Physiol Heart Circ Physiol 279:H130–H138

Mennone A, Alvaro D, Cho W, Boyer JL (1995) Isolation of small polarized bile duct units. Proc Natl Acad Sci USA 92:6527–6531

Mignen O, Le Gall C, Harvey BJ, Thomas S (1999) Volume regulation following hypotonic shock in isolated crypts of mouse distal colon. J Physiol 515:501–510

Mintenig GM, Valverde MA, Sepulveda FV, Gill DR, Hyde SC, Kirk J, Higgins CF (1993) Specific inhibitors distinguish the chloride channel and drug transporter functions associated with the human multidrug resistance P-glycoprotein. Recept Channels 1:305–313

Nilius B, Droogmans G (2003) Amazing chloride channels: an overview. Acta Physiol Scand 177:119–147

Nilius B, Eggermont J, Voets T, Buyse G, Manolopoulos V, Droogmans G (1997) Properties of volume-regulated anion channels in mammalian cells. Prog Biophys Mol Biol 68:69–119

Park JS, Choi YJ, Vicki J. Siegrist VJ, Ko YS, Won Kyoo Cho WK (2007) Permissive role of calcium on regulatory volume decrease in freshly isolated mouse cholangiocytes. Pflugers Arch Eur J Physiol 455:261–271

Roman RM, Wang Y, Fitz JG (1996) Regulation of cell volume in a human biliary cell line: activation of K^+ and Cl^- currents. Am J Physiol 271:G239–G248

Schmid A, Blum R, Krause E (1998) Characterization of cell volume–sensitive chloride currents in freshly prepared and cultured pancreatic acinar cells from early postnatal rats. J Physiol 513(pt 2):453–465