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Swelling-activated Chloride and Potassium Conductance in Primary Cultures of Mouse Proximal Tubules. Implication of KCNE1 Protein

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Abstract. Volume-sensitive chloride and potassium currents were studied, using the whole-cell clamp technique, in cultured wild-type mouse proximal convoluted tubule (PCT) epithelial cells and compared with those measured in PCT cells from null mutant *kcne1* −/− mice. In wild-type PCT cells in primary culture, a Cl^- conductance activated by cell swelling was identified. The initial current exhibited an outwardly rectifying current-voltage (*I-V*) relationship, whereas steady-state current showed decay at depolarized membrane potentials. The ion selectivity was $I^- > Br^- > Cl^- >>$ gluconate. This conductance was sensitive to 1 mM 4,4′-Diisothiocyanostilbene-2,2′-disulfonic acid (DIDS), 0.1 mM 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) and 1 mM diphenylamine-2-carboxylate (DPC). Osmotic stress also activated K^+ currents. These currents are time-independent, activated at depolarized potentials, and inhibited by 0.5 mM quinidine, 5 mM barium, and 10 μM clofilium but are insensitive to 1 mM tetraethylammonium (TEA), 10 nM charybdotoxin (CTX), and 10 μM 293B. In contrast, the null mutation of *kcne1* completely impaired volume-sensitive chloride and potassium currents in PCT. The transitory transfection of *kcne1* restores both Cl^- and K^+ swelling-activated currents, confirming the implication of KCNE1 protein in the cell-volume regulation in PCT cells in primary cultures.

Key words: Cell volume — *kcne1* — Kidney — Chloride conductance — Potassium conductance

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Introduction

KCNE1 (IsK), a small 129-amino-acid protein with a single putative membrane domain, is expressed in many mammalian tissues: the inner ear (Vetter et al., 1996), the heart, lung, testis, ovaries, small intestine, peripheral blood leukocytes (Chouabe et al., 1997), the stomach, pancreas (Demolombe et al., 2001) and the kidney (Takumi, Ohkubo & Nakanishi, 1988; Chouabe et al., 1997; Demolombe et al., 2001). When expressed in *Xenopus laevis* oocytes (Takumi et al., 1988) or mammalian HEK 293 cells (Freeman & Kass, 1993), KCNE1 protein induces a slowly activating, outwardly rectifying, voltage-gated potassium current. Further experiments have shown that this current is due to the association of KCNE1 protein with KCNQ1 (KVLQT1) K^+ channel protein (Barhanin et al., 1996).

In order to analyze the in vivo function of the KCNE1 protein, mice carrying the null mutation of the *kcne1* gene were engineered. Homozygous null mutant mice are deaf and exhibit classic shaker/waltzer behavior (Vetter et al., 1996). So far, a functional role for KCNE1 has been proposed in the heart and in the inner ear (Vetter et al., 1996).

In the kidney, KCNE1 protein has been shown to be confined to the apical membrane of epithelial cells in the proximal tubule (Sugimoto et al., 1990; Vallon et al., 2001). Vallon et al. indicated that KCNE1-dependent K^+ movement from the cell to the lumen contributes to the maintenance of the electrical driving force for Na^+-coupled transport in the proximal tubule (Vallon et al., 2001). However, the pore-forming partner(s) of KCNE1 and the role of the respective channel complex in the kidney are not known. In proximal tubule cells, as in most cells, an increase in cell volume is followed by regulatory volume decrease (RVD) mediated by efflux of Cl^- and K^+ through swelling-activated Cl^- and K^+

channels. Interestingly, KCNE1 has been shown to act as a potent channel activator able to interact with endogenous K^+ and Cl^- channels (Attali et al., 1993; Ben-Efraim, Shai & Attali, 1996), strongly suggesting that KCNE1 and phospholemman are activators of the same endogenous Cl^- channel. Phospholemman has also been shown to regulate volume-sensitive Cl^- channels (Moorman et al., 1992). Moreover, other studies have reported that regulatory volume decrease (RVD) could involve the KCNE1 protein (Wangemann et al., 1995) and that the potassium channel complex KCNQ1/KCNE1 plays an important role in the RVD process (Lock & Valverde, 2000). Therefore, the implication of KCNE1 in RVD regulation in other tissues led us to examine whether the same implication might occur in the proximal tubule. For this purpose, we decided to analyze swelling-activated Cl^- and K^+ currents in primary cultures of proximal tubules microdissected from kidney of either control (*kcne1* +/+) or KCNE1 knock-out mice (*kcne1* −/−).

Materials and Methods

Animals

Knockout KCNE1 mice were generated by the gene-targeting methodology already described (Vetter et al., 1996). The mutation has been maintained on the 129/Sv genetic background and all the animals used in this study, *kcne1* (−/−) and WT, were inbred 129 Sv. Mice were allowed free access to food and water in a facility (at 21 ± 1°C with 12-hour light/dark cycles) monitored by Institut de Pharmacologie Moléculaire et Cellulaire staff in full compliance with the French government animal welfare policy.

Primary Cultures

Proximal tubules were microdissected under sterile conditions from kidneys obtained from 4- to 5-week-old male mice killed by cervical dislocation. Kidneys were perfused with Hank's solution (Gibco) containing 700 kU/l collagenase (Worthington) and were cut into small pyramids, which were incubated one hour at room temperature in the perfusion buffer containing 160 kU/l collagenase, 1% Nuserum, and 1 mM $CaCl_2$ and were continuously aerated. The pyramids were then rinsed thoroughly in the same buffer devoid of collagenase. The individual nephrons were dissected by hand in this buffer under binoculars, using stainless steel needles mounted on Pasteur pipettes. After rinsing in the dissecting medium, tubules were transferred to collagen-coated 35-mm Petri dishes filled with a culture medium composed of equal quantities of DMEM and Ham F12 (Gibco), containing 15 mM $NaHCO_3$, 20 mM HEPES pH 7.5, 1% serum, 2 mM glutamine, 5 mg/l insulin, 50 nM dexamethasone, 10 μg/l epidermal growth factor, 5 mg/l transferrin, 30 nM sodium selenite, 10 nM triiodothyronine. Cultures were maintained at 37°C in a 5% CO_2/95% air water-saturated atmosphere. The medium was changed 4 days after seeding and then every two days.

mRNA Expression of *kcne1* and *kcnq1*

RT-PCR was used to verify the expression of KCNE1 and KCNQ1 mRNAs. Total RNA was isolated from 10-day-old primary cultures of proximal tubules dissected from *kcne1* −/− and *kcne1* +/+ kidney mice. Total RNA (2 μg) was reverse-transcribed and 25% of the product reaction was amplified by PCR with the following specific primers. KCNE1 forward primer: 5′–CGA-CTG-TTC-TGC-CCT-TTC-TG–3′. Reverse primer: 5′–CTC-AGT-GGT-GCC-CCT-ACA-AT–3′, product size: 453pb. KCNQ1 forward primer: 5′–CTG-AGA-AAG-ATG-CGG-TGA-AC–3′. Reverse primer: 5′–TGG-GGG-TCA-GCA-GTG-TCT-CC–3′, product size: 700 bp. PCR conditions were 40 cycles of 25 s at 94°C, 25 s at 58°C and 30 s at 72°C. After electrophoresis on agarose gel, *kcne1*- and *kcnq1*-amplified fragments were transferred onto nylon membranes and then probed at high stringency with ^{32}P- labeled DNA fragments of KCNE1 (sense bp 98–117, antisense bp 528–547 from GenBank NMU008424, Tm 62°) and KCNQ1 (sense bp 756–775, antisense bp 1387–1406 from GenBank MMU 70068, Tm 62°).

Expression in Cultured Cells

The cDNA encoding *kcne1* was introduced into a polycistronic expression vector derived from the pIRESneo plasmid (CMV promoter; Clontech), in which the neomycin resistance gene was replaced by cDNA encoding the chain of the human CD8 cell surface antigen. Distal cells were transfected using the DAC-30 method according to the manufacturer's instructions (Eurogentec, Herstal, Belgium). Six-day-old cultured cells grown on 35 mm diameter Petri dishes were serum-starved 24 hours before transfection. Cells transfected with 2 μg of *CD8-kcne1* coexpress KCNE1 and CD8 at their plasma membrane and can be visualized using anti-CD8 antibody-coated beads (Dynabeads® M-450 manufactured by Dynal ASA, Oslo, Norway) (Jurman et al., 1994). Cells were tested electrophysiologically 48 hours after transfection.

Cell Volume Measurements

Cell Volume Measurement with Fluorescent Probe

The relative cell volume was monitored by image analysis with Fura 2 as fluorescent volume indicator, as previously reported (Rubera et al., 1997b). Twelve- to fifteen-day-old PCT monolayers grown on Petri dishes were loaded with a solution of 2 μM fura 2 containing 0.01% pluronic acid for 20 min at 37°C and were then washed with an NaCl solution. The fluorescence was monitored with 360-nm excitation wavelength. At 360 nm, the variations of the signal emitted by the probe are directly proportional to the variations of the cell volume. In a typical experiment, the cells were first perfused with an isotonic NaCl solution containing (in mM) 110 NaCl, 5 KCl, 1 $CaCl_2$, 90 mannitol, and 10 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid pH 7.4, [osmotic pressure (Posm) = 300 mosmol/kg H_2O] at 30 ml/min, and images were averaged 8 times and recorded every 5 s for 10 min. Once the fluorescence had been stabilized, a hypotonic shock was induced by perfusing the NaCl solution without mannitol (Posm = 200 mosmol/kg H_2O). The estimation of relative change in cell volume from the fluorescent signal was made assuming that a 30% decrease in the osmolarity caused a decrease of the fluorescent signal corresponding to a maximum swelling of 30% compared with the initial volume. The means of relative volume changes were obtained by the analysis of 5–10 zones in each of *n* number of cultures chosen with the software. Each zone delimited a cytoplasmic area chosen in individual cells.

Image Analysis

The optical system was composed of a Zeiss ICM-405 inverted microscope and a Zeiss 40× objective, which was used for epiflu-

Table 1. Composition of solutions used in whole-cell clamp experiments

	Cl^- Substitution	K^+ Substitution	NMDGCl	Nagluconate	Pipette	
					NMDGCl	Kgluconate
Na^+	0, 70 or 130	15, 70 or 135	–	135	–	–
K^+	–	125, 70 or 5	–	5	–	125
Ca^{2+}	1	1	1	1	–	–
$NMDG^+$	140, 70 or 10	–	140	–	140	–
Cl^+	140, 70 or 10	2	142	2	140	20
Gluconate	70 or 130	140	–	140	–	105
Glucose	5	5	5	5	–	–
Mg-ATP	–	–	–	–	5	5
EGTA	–	–	–	–	5	5
HEPES	10	10	10	10	10	10
Mannitol			0 or 60	0 or 60		
Osmolarity (mosm/kg)	290	290	290 or 350	290 or 350	290	290

Concentrations are given in mM. Bath solutions were titrated to pH 7.4 with NaOH. Pipette solutions were titrated to pH 7.4 with tris(hydroxymethyl)aminomethane. Solutions were prewarmed to 37°C. Solutions were made hyperosmotic by addition of mannitol.

orescent measurements with a 75-W xenon lamp. The excitation beam was filtered through a narrow-band filter centred at 360 nm and mounted in a motorized wheel (Lambda 10-2; Sutter Instrument) equipped with a shutter to control the exposure times. The incident and the emitted fluorescence radiation were separated through a Zeiss chromatic beam splitter. Fluorescence emission was selected through a 510-nm narrow-band filter (Oriel). The transmitted light images were viewed by an intensified camera (Extended ISIS; Photonic Science, Sussex, UK). The 8-bit extended-ISIS camera was equipped with an integration module to maximize signal-to-noise ratio. The video signal from the camera proceeded to an image processor integrated in a DT2867 image card (Data Translation) installed in a Pentium 100 PC. The processor converts the video signal into 512 lines by 768 square pixels per line by 8 bits per pixel. The 8-bit information for each pixel represents one of the 256 possible grey levels, ranging from 0 (for black) to 255 (for white). Image acquisition and analysis were performed by the 2.0 version of AIW software (Axon Instrument). The final calculations were made using the Excel software (Microsoft).

Whole-Cell-Clamp Experiments

Whole-cell currents were recorded from 12- to 22-day-old cultured cells grown on collagen-coated supports maintained at 33°C for the duration of the experiments. The ruptured-patch whole-cell configuration of the patch-clamp technique was used. Patch pipettes (resistance 2–3 MΩ) were made from borosilicate capillary tubes (1.5 mm OD, 1.1 mm ID, Popper Manufacturing, Long Island City, N.Y), using a two-stage vertical puller (PP 83, Narishige, Tokyo) and filled with appropriate solutions (Table 1). Osmolarity of pipette solutions was adjusted to 290 mosm/kg H_2O, and pH for both solutions was maintained at 7.4. Cells were observed using an inverted microscope, whose stage was equipped with a water robot micromanipulator (WR 89, Narishige). The patch pipette was connected via an Ag/AgCl wire to the headstage of an RK 400 patch amplifier (Biologic). After formation of 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 capacitance. The membrane was ruptured by additional suction to achieve the conventional whole-cell configuration. The cell capacitance (C_m) was compensated by using settings available on the RK 400 amplifier. Membrane capacitance (C_m) of 20 cells was 18.7 ± 1.3 pF for *kcne1*−/− and 20.1 ± 1.5 pF for *kcne1*+/+ cells and did not differ significantly ($p < 0.05$, Student's test, NS paired *t*-test). With NMDG-Cl in bath and the pipette, the series resistance (R_s) averaged 12.8 ± 1 MΩ for *kcne1*+/+ cells ($n = 30$) and 11.3 ± 2.3 MQ ($n = 25$) for *kcne1*−/− cells. With potassium gluconate in the pipette and Na Gluconate in the bath, the R_s averaged 8.7 ± 0.9 MΩ ($n = 30$) for *kcne1*+/+ and 9.4 ± 1.3 MΩ ($n = 20$) for *kcne1*−/−. Whatever the cell type, in C^- or K^+ conditions, the series resistances (R_s) were not significantly different ($p < 0.05$, Student's test, NS paired *t*-test). Cell membrane potentials were measured at zero membrane current in the current-clamp mode of the amplifier. No series resistance compensation was applied, but experiments in which the series resistance was higher than 20 MΩ were discarded. Solutions were perfused in the extracellular bath using a four-channel glass pipette, the tip of which was placed as close as possible to the clamped cell.

Data Acquisition and Analysis

Voltage-clamp commands, data acquisition and data analysis were controlled via a computer equipped with a Digi data 1200 interface (Axon Instruments, Foster City, CA). pCLAMP software (versions 5.51 and 6.0, Axon Instruments) was used to generate whole-cell *I-V* relationships, with the membrane currents resulting from voltage stimuli filtered at 1 kHz, sampled at 2.5 kHz and stored directly on the computer hard disk. Cells were held at a holding potential (V_{hold}) of −50 mV, and 400 ms pulses from −100 to +120 mV were applied in increments of 20 mV every 2 s.

Chemicals

5-Nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) from Calbiochem was prepared at 100 mM in DMSO and used at 100 μM in final solutions. Diphenylamine-2-carboxylate (DPC) from Aldrich was prepared as 1 M stock solution in DMSO and dissolved at 1 mM in incubation medium. 4-4′-diisothiocyanostilbene-2,2′ disulfonic acid (DIDS) from Sigma was directly dissolved at a final concentration of 1 mM. 293B and clofilium were a gift from Dr. Barhanin (UMR CNRS 6097) and was prepared at 10 mM in DMSO and used at 10 μM in final solutions. Barium chloride from Merck was dissolved at a final concentration of 5 mM. All other products were from Sigma.

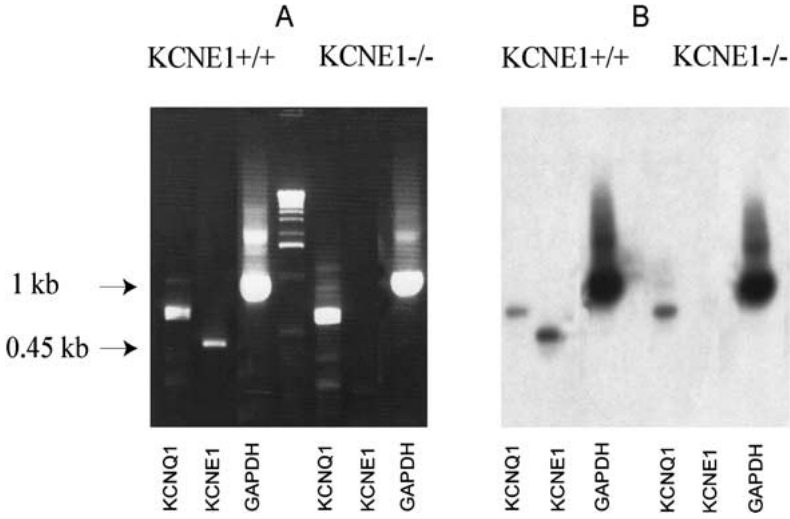

Fig. 1. Detection of transcripts encoding the *kcne1* and *kcnq1* sequence in primary cultures of PCT cells from *kcne1* +/+ and *kcne1* −/− mice. (*A*) KCNQ1 and KCNE1 transcripts were assessed by RT-PCR of total RNA extracted from 10-day-old primary cultures of murine PCT cells. Primers and protocol are reported in Material and Methods. (*B*) Southern probing was used in a second set of experiments to detect amounts of PCR products. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to estimate total mRNA amount.

Results

Identification of Transcripts Encoding the KCNQ1 and KCNE1 Sequence in Cultured PCT Cells from *kcne1* +/+ and *kcne1* −/− mice

Expression of both KCNQ1 and KCNE1 was assessed by reverse transcription of total RNA extracted from primary cultures of murine PCT cells. As illustrated in Fig. 1*A*, transcripts of KCNQ1 were detected in PCT cells from both *kcne1* +/+ and *kcne1* −/− mice, whereas transcripts of KCNE1 were only found in *kcne1* +/+ mice. The PCR products obtained from cultured PCT were analyzed further using Southern blot experiments. This analysis yielded the predicted bands for KCNQ1 and KCNE1 in PCT cells from wild type mice and confirmed the absence of KCNE1 in PCT cells from knock-out mice (Fig. 1*B*).

Influence of Hypotonic Shock on Relative Cell Volume

In previous works (Busch et al., 1992; Wangemann et al., 1995) it was postulated that KCNE1 could play a role in cell volume regulation. Therefore we studied this possibility in our cell models. For this purpose, relative cell volume was determined in monolayers by fluorescence video microscopy. The data are illustrated in Fig. 2. As expected, the reduction of osmolarity of the perfused solution caused a rapid increase in relative cell volume. This cell swelling was followed by an RVD in PCT cells from *kcne1* +/+ mice. Two minutes after the hypotonic shock, the relative cell volume reached 130.1 ± 2% (n = 3 monolayers) of the initial volume and returned to 105.2 ± 1% (n = 3 monolayers) of the original volume within 4 min. In contrast, the RVD phenomenon was completely impaired in cells originating from *kcne1* −/− mice: when perfused with the hypotonic solution, these cells never returned to their initial volume even after 20 minutes (*data not shown*).

In *kcne1* +/+ cells, the efficient RVD process was completely blocked in the presence of NPPB (100 μM) or quinidine (0.5 mM), indicating that Cl^- and K^+ channels were probably involved in RVD.

Cl^- Currents Induced by Osmotic Shock in Cultured PCT Cells from *kcne1* +/+ and *kcnel* −/− Mice

The effect of osmotic swelling on Cl^- conductance was tested using whole-cell-clamp analysis. Whole-cell currents were recorded with Ca^{2+}-free pipette solutions containing NMDG chloride in the pipette and in the extracellular solution (Table 1). The control currents were recorded with a pipette solution isotonic (290 mosm/kg H_2O) as compared to the osmolarity of the bath medium (350 mosm/kg H_2O), then a hypotonic shock was induced by reducing the osmolarity of the bath medium to 290 mosm/kg H_2O. Voltage-clamp experiments were performed holding the cell at −50 mV and applying voltage steps of 400 ms duration every 2 s from −100 to 120 mV in 20-mV increments.

Cl^- Currents in kcne1 +/+ *Cells*

The results obtained when the osmotic shock was performed according to the first experimental protocol are reported in Fig. 3. Figure 3*A* and 3*B* show families of currents recorded in control conditions and 4–5 minutes after the beginning of osmotic shock (recording time). In a large majority of cells, the voltage step protocol elicited small currents (Fig. 3A) with a reversal potential of −0.3 ± 1.5 mV (n = 20 cells from 9 monolayers), indicating that Cl^- may well be the charge carrier. The amplitude of the cur-

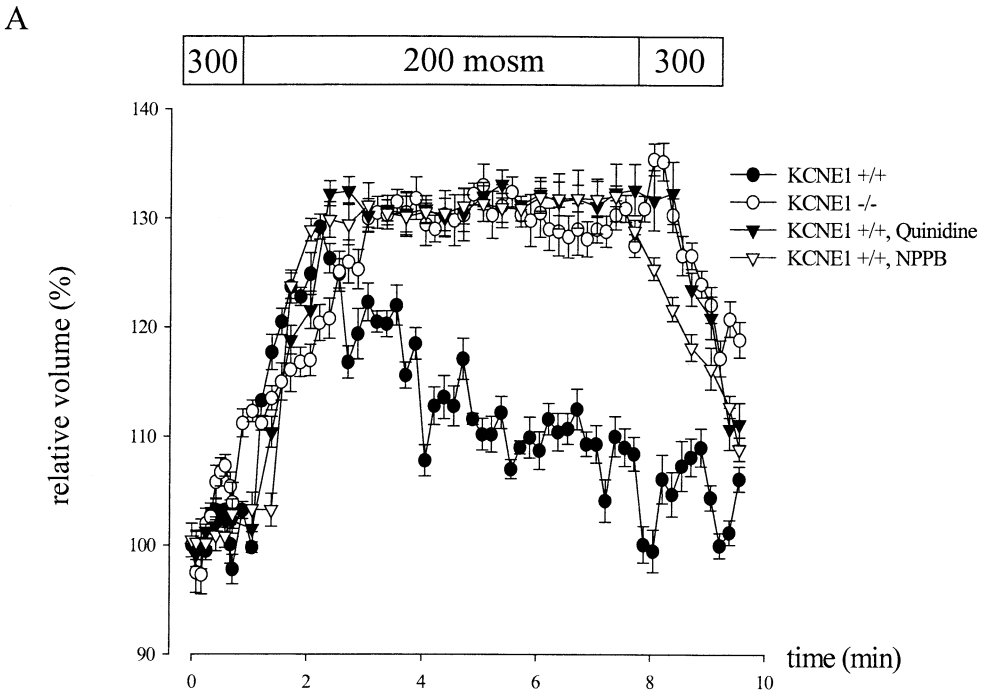

Fig. 2. Regulatory volume decrease in PCT cells from *kcne1*+/+ and *kcne1*−/−. Effect of a hypotonic shock on primary cultured cells. After loading with fura 2, cultures were rinsed in 300 mosmol/kg H_2O NaCl solution, and fluorescence of fura 2 was measured at 360 nm for a control period of 1 min. Then a hypotonic shock was induced by perfusing a 200 mosmol/kg H_2O NaCl solution. Images were recorded every 5 s. After analysis, relative volume change in percent of initial volume was plotted against time as explained in Methods. Measurements were performed on 10 monolayers (25 random cells each) from *kcne1*−/− and 3 monolayers (25 random cells each) from *kcne1*+/+.

rents was 246 ± 54 pA (n = 20 cells from 9 monolayers) at +80 mV. In more than 95% of the *kcne1* +/+ cells, an increase in the whole-cell current was observed within 4–5 minutes after perfusion with a 290 mosm/kg H_2O solution: the initial currents, measured 20 ms after the onset of the voltage pulse, rectified in the outward direction (Fig. 3*B*). They reversed at −3 ± 1.2 mV (n = 20 cells from 9 monolayers) and the total current at 80 mV was 3.4 times the current at −80 mV (80 mV = 1989 ± 238 pA; −80 mV = −586 ± 76 pA, n = 20 cells from 9 monolayers). The *I-V* relationships are illustrated in Fig. 3*F*. These large, outwardly rectifying currents showed time-dependent inactivation at depolarizing step potentials ≥60 mV. These data were obtained from experiments performed in symmetrical Cl^- concentrations. The reversal potential was very close to that of Cl^- and, in the absence of permeant cations in the pipette, the outward current was carried by Cl^-

When the cells were exposed to hyperosmotic solution (Fig. 3*C*), the currents returned to the control level within 3–4 minutes (% inhibition at +80 mV = 80 + 7.4%, n = 18 cells from 4 monolayers).

To study the anion permeability of the cell membrane, all except 2 mM of the Cl^- in the bath solution was replaced with I^- or Br^-. Table 2 summarizes the reversal potentials as well as the calculated permeability ratios obtained for a given anion. In the presence of each anion, the reversal potential shifted towards negative values. Replacing internal chloride with gluconate shifted the reversal potential towards negative voltages (Table 2). The sequence for this conductance was $I^- > Br^- > Cl^- >>$ gluconate.

To further characterize the Cl^- current, we tested three anion channel blockers added separately to the bathing solution. As shown in Fig. 4, NPPB (0.1 mM), and DPC (1 mM) and DIDS (1 mM) strongly inhibited the swelling-activated Cl^- current measured at E_m = −80 mV or + 80 mV.

To prove that under these conditions only Cl^- currents were recorded, we measured the reversal potential shifts induced by changing $[Cl^-]_e$. The results are reported in Fig. 5. Three extracellular Cl^- concentrations were tested successively: 140 mM (Fig. 5*A*), 70 mM (Fig. 5*B*) and 10 mM (Fig. 5*D*). Under these experimental conditions, the theoretical values of reversion potentials for Cl^- were, respectively: 0 mV, 17.5 mV and 66.5 mV. After correction of the liquid junction potential, the E_{rev} were 1.6 ± 1.2 mV (n = 8) for 140 mM Cl^-, 18.6 ± 0.9 mV (n = 8) for 70 mM Cl^- and 65 ± 1.1 mV (n = 8) for 10 mM Cl^- (Fig. 5*E*). These results are in good agreement with the theoretical values calculated with the Nernst equation. Finally, once

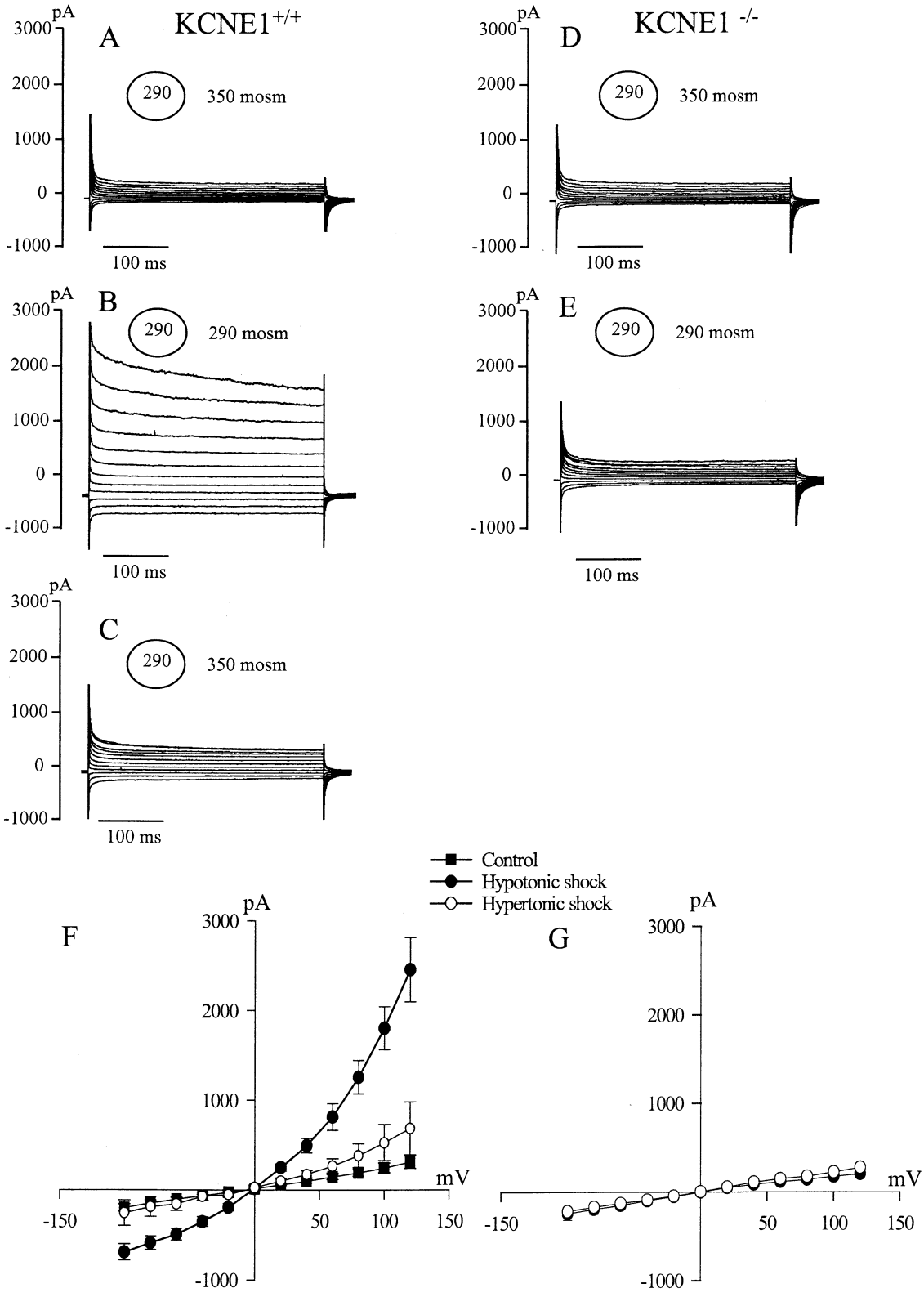

Fig. 3. Characteristics of swelling-activated whole-cell Cl^- currents in PCT cells in primary cultures of *kcne1*+/+ and *kcne1*−/− mice activated by an osmotic shock. Whole-cell currents were recorded with an isotonic solution containing 140 mM NMDGC1 in the pipette and in extracellular bath. Membrane voltage was held at −50 mV and stepped to test potential values between −100 mV to 120 mV in 20 mV increments. (*A*) and (*D*) Whole-cell currents recorded in control conditions (350 mosm/kg H_2O) in extracellular bath after the mechanical rupture of membrane. Whole-cell currents were recorded after 4–5 min extracellular perfusion of a 290 mosmol/kg H_2O hypotonic solution (*B*) on PCT cells from *kcne1*+/+ and (*E*) on PCT cells from *kcne1*−/−. (*C*) Effects of a hyperosmotic solution (350 mosm/kg H_2O) on swelling-induced whole-cell Cl^- currents. Average current-voltage relationships measured at 20 ms after onset of pulse for KCNE1+/+ (*F*) and KCNE1−/− (*G*). Each value is mean ± SE of *n* cells obtained from 9 monolayers.

Table 2. Effect of substitution of Cl^- by various anions on E_{rev}

Anion	*n*	E_{rev} (mV)	P_X/P_{Cl}
Extracellular I^-	8	−9.7 ± 1.9	1.42 ± 0.08
Extracellular Br^-	5	−6.5 ± 1.3	1.28 ± 0.06
Intracellular gluconate	6	−50.3 ± 4.0	0.19 ± 0.03

Values are means ± SE of *n* cells. E_{rev} was calculated for initial currents extrapolated at $t = 20$ ms using exponential fitting of the current decay. The estimated relative permeability P_X/P_{Cl} was calculated using the equation: $E_{rev} = (RT/zF)$ log $((([Cl^-]_o P_{Cl} + [X]_o P_X)/([Cl^-]_i P_{Cl} + [X]_i P_X))$.

$[Cl^-]_e$ changes had been performed, re-exposing the same cells to 140 mM Cl^- produced currents identical to those recorded initially (Fig. 5*C*).

Cl^- Currents in kcne1 −/− *Cells*

In this group, the cells never exhibited a significant increase of their swelling Cl^- conductances (Fig. 3*D*, *E*, *G*). Figure 3*D*, *E* gives recordings of the currents obtained in control conditions and 4–5 minutes after the beginning of osmotic shock (Table 1). Figure 3*G* shows that the voltage-step protocol elicited small currents that changed linearly with the membrane potential in *kcne1* −/− PCT cell cultures. In control conditions, the amplitude of the current recorded at +80 mV was 140 + 23 mV, and the E_{rev} was −0.5 ±1.1 mV ($n = 25$ cells from 9 monolayers). A three-minute exposure of the cells to the osmotic shock did not modify the currents. The currents recorded 4–5 minutes after the beginning of osmotic shock reversed at −1.4 ± 1.9 mV with an amplitude recorded at +80 mV of 175 ± 34 pA ($n = 25$ cells from 9 monolayers).

The sensitivity of this small current to NPPB, DPC and DIDS was also tested. As illustrated in Fig. 4*B*, exposure of the cells to standard solutions in the presence of 0.1 mM NPPB or 1 mM DPC inhibited both initial inward and outward currents. The effect of 1 mM DIDS was voltage dependent.

K^+ Currents Induced by Osmotic Shock in Cultured PCT Cells from *kcne1* +/+ and *kcne1* −/− Mice

The effect of osmotic swelling on K^+ conductance was tested in the presence of 0.1 mM NPPB. The first experimental series was carried out with 5 mM potassium in the bath solution and 125 mM potassium and 5 mM EGTA in the pipette (Table 1). In these conditions, the theoretical E_{rev} is −81 mV.

K^+ Currents in kcne1 +/+ *Cells*

Figure 6*A* illustrates the family of current recordings in *kcne1*+/+ cells, made in a hypertonic bath medium with test potentials that ranged from −100 mV to 120 mV in increments of 20 mV. The *I-V* curve was measured 200 ms after onset of the pulse. Small outward currents were slightly increased during positive voltage pulses. The conductance measured by the maximal slope of the *I-V* curve averaged 1.7 ± 0.3 nS ($n = 9$). As illustrated in Fig. 6*B*, outward currents were activated when the hypertonic bath solution was replaced by an isotonic solution of identical ionic composition. Maximal current activation was reached five minutes after the onset of the osmotic shock. The *I-V* curve of Fig. 6*F* shows that the activation of outward currents became significantly different from 0 at −60 mV (volume-activated current: 30 ± 11 pA, $n = 9$) and increased with depolarizing potentials. The currents reversed at −77 ± 8 mV, indicating that they were carried by K^+ ions. The calculated maximal slope conductance increased significantly (24.7 ± 1.2 nS, $p < 0.0001$, $n = 9$) as compared to control values (*see above*). Finally, re-exposing the same cells to hypertonic solution induced a decrease in the amplitude of the outward current within 2–3 min (Fig. 6*C*). However the current amplitude returned to values slightly higher than that initially measured. The reversal potential remained close to that of K^+ ions (−74.4 ± 1.5 mV, $n = 9$).

Swelling-activated K^+ conductance was then analyzed following the addition of different K^+ channel blockers added to the isotonic bath solution. Figure 7 gives the percentage of inhibition of the swelling-activated outward current measured at +80 mV. To test the possibility that the K^+ swelling current could belong to the KCNQ1/KCNE1 potassium channel complex, we used chromanol 293B, which is a specific inhibitor of KCNQ1 K^+ channels (Warth et al., 1996). Application of 293B (10 μM) before and during the osmotic shock did not modify the outward current at + 80mV (control: 989 ± 142 pA; 293B: 1015 ± 254 pA, $n = 5$ monolayers, NS, paired *t*-test) and the maximal slope conductance (control: 15.9 ± 0.8 nS; 293B: 14.1 ± 1.1 nS, $n = 5$, NS, paired *t*-test). Surprisingly, clofilium (10 μM), which is also an inhibitor of KCNQ-family K^+ channels, strongly blocked the swelling-activated K^+ conductance by 55 ± 5% ($n = 5$)

Quinidine (0.5 mM) and barium (1 mM) also inhibited the swelling-activated K^+ current at + 80 mV by 64 ± 5.5% ($n = 6$) and 60 ± 8% ($n = 8$), respectively (Fig. 7). In contrast, TEA (1 mM) was inefficient in blocking outward currents since the % of inhibition at + 80 mV was 5 ± 2 % ($n = 8$) only. Among the inhibitors of K^+ channels, charybdotoxin (CTX) was found to be a very potent blocker of Ca^{2+} -sensitive K^+ currents. In the present study, the addition of CTX (10 nM) to the bath medium did not significantly modify the swelling-activated K^+ conductance (Fig. 7).

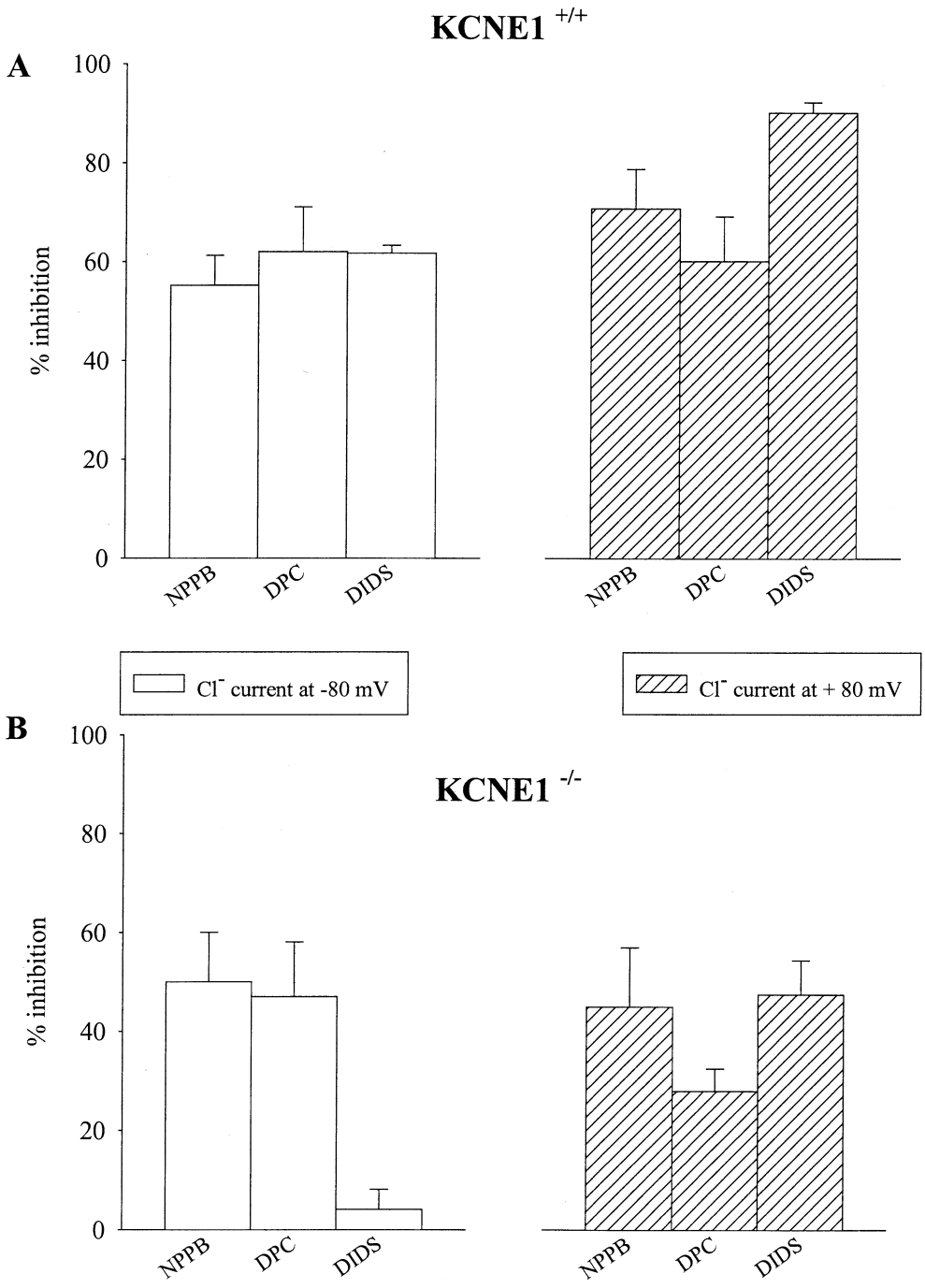

Fig. 4. Histograms of % inhibition of different Cl^- channel inhibitors on swelling-activated Cl^- currents in PCT cells from *kcne1*+/+ and *kcne1*−/− cells. Whole-cell currents were recorded with an isotonic solution containing 140 mM NMDGCl in the pipette and in the extracellular bath. Membrane voltage was held at −50 mV and stepped to test-potential values between −100 mV to 120 mV in 20-mV increments. Whole-cell currents were recorded after 4–5 min extracellular perfusion of a 290 mosmol/kg H_2O hypotonic solution in the presence of 1 mM DIDS, 0.1 mM NPPB or 1 mM DPC. Values measured 20 ms after the onset of pulse at +80 mV or at −80 mV are converted to % inhibition. (*A*) Effects of Cl^- inhibitors on swelling-activated Cl^- currents in PCT cells from *kcne1* +/+. (*B*) Effects of Cl^- inhibitors on swelling-activated Cl^- currents in PCT cells from *kcne1* −/−. Each value is mean ± SE of *n* cells obtained from 3 monolayers.

To characterize further the K^+ currents, we tested the Nernstian shift by changing the extracellular concentration of K^+ from 5 mM (Fig. 8A) to 70 mM (Fig. 8*B*) or 125 mM (Fig. 8*C*). After correction of the liquid junction potential, the E_{rev} recorded were −82 ± 3 mV (n = 5) for 5 mM K^+, −17 ± 4 mV (n = 5) for 70 mM K^+ and −1.4 ± 2.1 mV (n = 5) for 125 mM K^+ (Fig 8*D*). As expected, these values

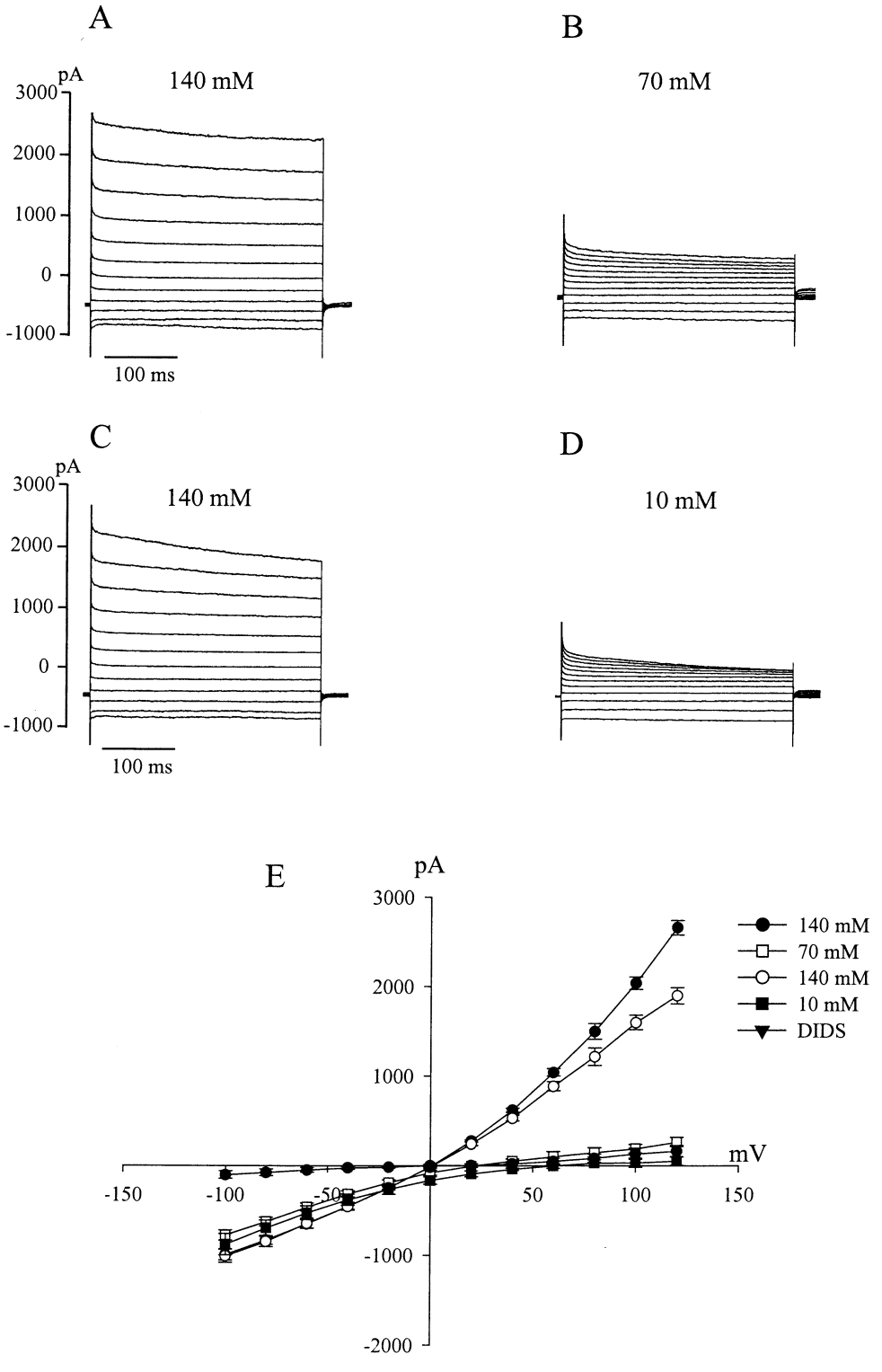

Fig. 5. Swelling-activated whole-cell Cl^- currents recorded in primary cultures of PCT from *kcne1* +/+ mice in the presence of different external Cl^- concentrations. Whole-cell currents were recorded with an isotonic solution containing 140 mM NMDGCl in the pipette and 140 mM, 70 mM or 10 mM $NMDGCl^-$ in the extracellular bath. Membrane voltage was held at −50 mV and stepped to test potential values between −100 mV to 120 mV in 20-mV increments. Whole-cell currents were recorded after 4–5 min extracellular perfusion of a 290 mosmol/kg H_2O hypotonic solution: (*A*) in the presence of 140 mM Cl^-, (*B*) 70 mM Cl^- and (*D*) 10 mM Cl^- in the extracellular bath. (*C*) Whole-cell currents recorded after re-exposure of the cells to 140 mM Cl^-. Average current-voltage relationships measured 20 ms after onset of pulse (*E*). Each value is mean ± SE of 8 cells obtained from 4 monolayers.

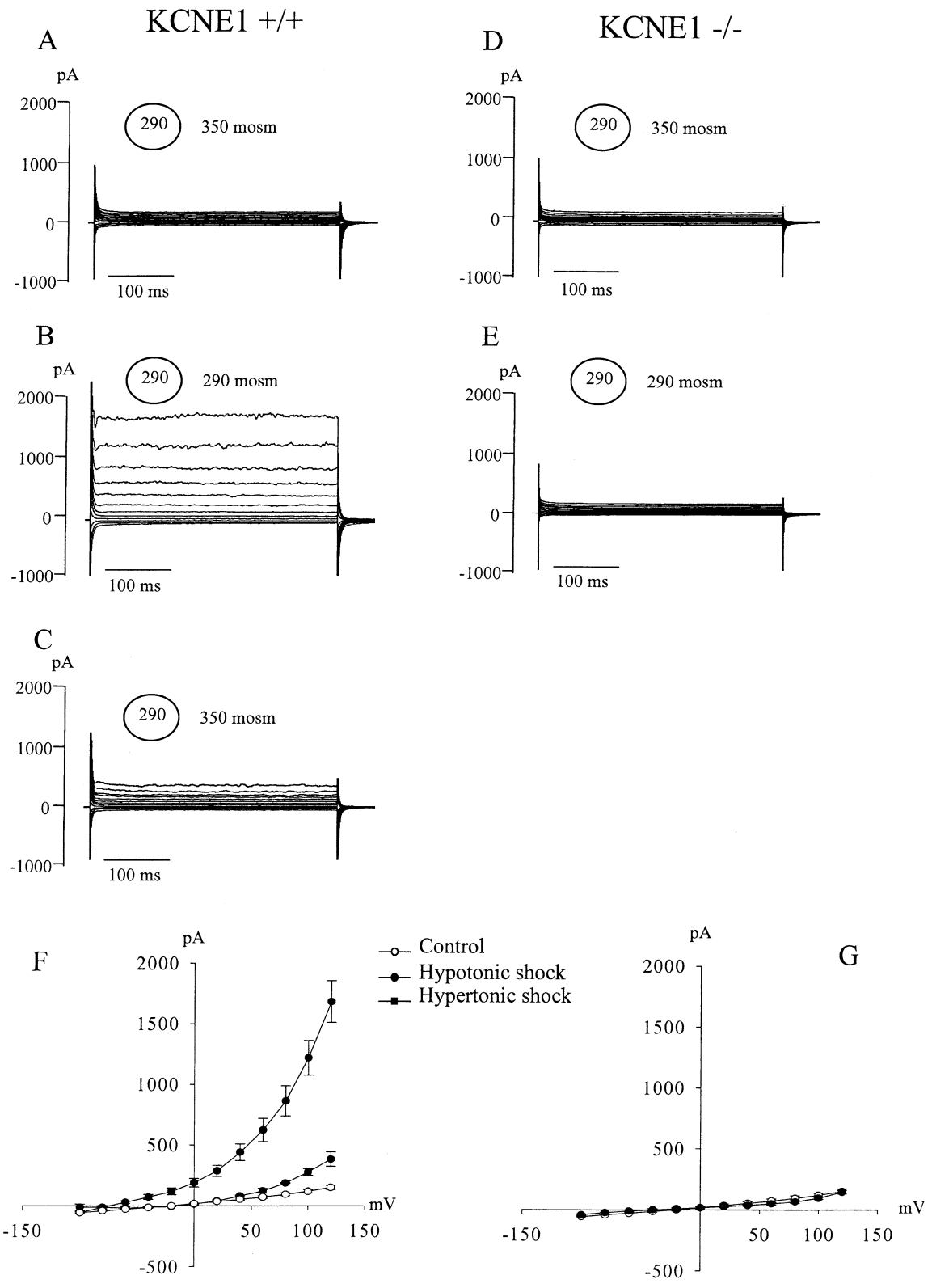

Fig. 6. Characteristics of swelling-activated whole-cell K^+ currents in PCT cells in primary cultures of *kcne1* +/+ and *kcne1*−/− mice. Whole-cell currents were recorded with an isotonic solution containing 125 mM of K^+ in the pipette and 5 mM of K^+ in the extracellular bath. Membrane voltage was held at −50 mV and stepped to test-potential values between -100 mV to 120 mV in 20-mV increments. (*A*) and (*D*) Whole cell currents recorded in control conditions (350 mosm/kg) in the extracellular bath after the mechanical rupture of membrane. Whole-cell currents were recorded after 4–5 min extracellular perfusion of a 290 mosmol/kg H_2O hypotonic solution (*B*) for PCT cells from *kcne1* +/+ and (*E*) for PCT from *kcne1* −/−. (*C*) Effects of a hyperosmotic solution (350 mosm/kgH_2O) on swelling-induced whole-cell K^+ currents. Average current-voltage relationships measured at 200 ms after onset of pulse, obtained from the same cell at rest (*F*). Values are means ± SE of *n* different monolayers from 4 different mice. (*G*) Values are means of *n* cells obtained from 16 mono layers from 4 different mice.

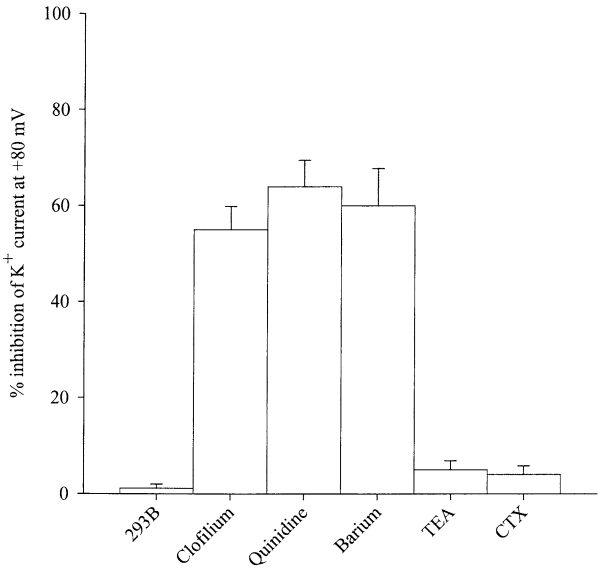

Fig. 7. Histogram of percent inhibition of different K^+ channel inhibitors on swelling-activated K^+ currents on PCT cells from *kcne1* +/+ mice. Membrane voltage was held at −50 mV and stepped to test potential values between −100 to +120 mV in 20-mV increments. Whole-cell currents were recorded after 4–5 minutes extracellular perfusion of a 290 mosmol/kg H_2O hypotonic solution in the presence of 10 μM 293B, 10 μM clofilium, 0.5 mM quinidine, 1 mM barium,1 mM TEA, or 10 nM charybdotoxin. Values measured 200 ms after the onset of the pulse at +80 mV are converted to percent inhibition. Each value is the mean ± SE of *n* cells obtained from 13 monolayers.

were close to the theoretical values given by the Nernst equation (−81 mV, −14.6 mV and 0 mV respectively).

K^+ *Currents in* kcne1 −/− *Cells*

As illustrated in Fig. 6*D* and 6*E*, in cultured proximal cells from *kcne1* −/− mice, the osmotic shock did not induce significant swelling-sensitive K^+ currents. The *I-V* curves of Fig. 6*G* show that the maximal slope conductances calculated in control conditions and during osmotic shock were low and not significantly different (control: 1.4 ± 0.4 nS; osmotic shock: 1.3 ± 0.1 nS, $n = 16$, NS, paired *t*-test). In both conditions, the E_{rev} for these small currents were for the control: −58.0 ± 3.9 mV and for the osmotic shock: −68.5 ± 3.2 mV, $n = 16$, NS, paired *t*-test. The inability of osmotic shock to trigger the development of K^+ currents was observed in 100 % of the trials.

Time Course of Cl^- and K^+ Current Activation by an Osmotic Shock in Cultured PCT Cells from *kcne1* +/+ and *kcne1* −/− Mice

In these experiments, the pipette solution was maintained at 290 mosm/kg H_2O. Figure 9*A* and 9*B* illustrate the time course of K^+ and Cl^- currents in both cell types. At an extracellular solution osmolarity of 350 mosm/kg H_2O (Table 1: solution NMDGC1 containing 60 mM mannitol), the voltage-step protocol elicited small currents. The monolayers were then perfused with a 290 mosm/kg H_2O solution. In *kcne1* +/+ cells, an increase in the whole-cell currents was observed within 1 minute. The currents reached a maximum after 5–6 minutes and remained stable for five minutes. For Cl^- currents, the maximum intensities were close to 1900 pA, whereas K^+ currents reached a maximum at 1500 pA. When the cells were re-exposed to hyperosmotic solution (350 mosm/kg H_2O), the currents returned to the control level within 2–3 minutes. Such variations in Cl^- and K^+ currents were never observed in monolayers from *kcne1* −/− mice (Fig 9*A* and 9*B*).

Cl^- and K^+ Currents Activated by an Osmotic Shock in Cultured PCT Cells from *kcne1* −/− Mice Transfected with *kcne1* cDNA

Kcne1 −/− PCT cells in primary culture were transfected with *pIres-CD8-kcne1* plasmid, allowing visualization of transfected cells using anti-CD8 antibody-coated beads. 48 hours after transfection, whole-cell currents of coated cells were recorded. Figure 10*A*, *B* represents Cl^- currents recorded in control conditions and 4–5 minutes after the beginning of osmotic shock. The control currents reversed at 1.3 ± 1.5 mV ($n = 6$) and the total current at +80 mV was 1.3 times the current at −80 mV (+80 mV = 192 ± 38 pA, −80 mV = −143 ± 3 pA; $n = 6$). The *I-V* relationship for initial currents is illustrated in Fig. 10*B*. 4–5 minutes after hypotonic shock, initial currents displayed an outward rectification and at this time, the initial current recorded at +80 mV was 2.6 times the current at −80 mV (+80 mV = 1008 ± 112 pA, −80 mV = −392 ± 71 pA; $n = 6$). These large outwardly rectifying currents showed time-dependent inactivation at depolarizing step potentials >60 mV. When cells were re-exposed to the hyperosmotic solution, the currents returned to the control level within 2–3 min (Fig. 10*B*). Overall, the Cl^- currents induced by osmotic shock at positive potentials closely resembled the swelling-activated Cl^- currents recorded in *kcne1* +/+ PCT cells.

In a second series of experiments, swelling-activated K^+ conductance was analyzed in transfected *kcne1* −/− cells. Figure 11*A* gives the recording of K^+ currents obtained in cells expressing the CD8 protein. In these cells, the osmotic shock induced outward K^+ currents that reversed at −72 ± 9 mV ($n = 5$) (Fig. 11*B*). These currents were strongly inhibited by application of a hypertonic solution (Fig. 11*A* and *B*). To verify whether K^+ currents identical to those recorded in wild-type cells were developed, we tested the effects of the K^+ blockers previously used. Quinidine (0.5 mM), barium (1 mM) and clofilium

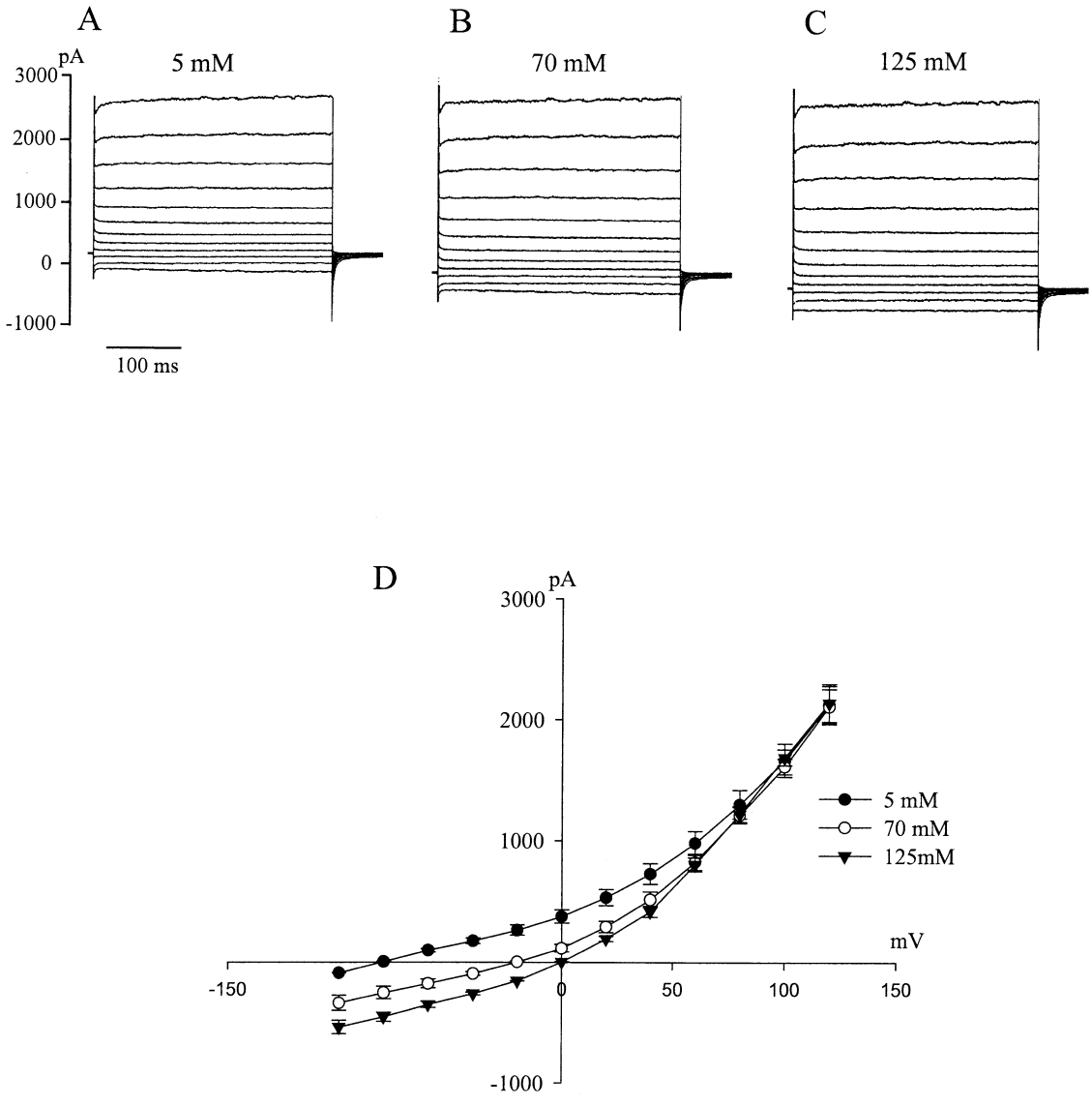

Fig. 8. Swelling-activated whole-cell K^+ currents recorded in primary cultures of PCT from *kcne1* +/+ mice in the presence of different external K^+ concentrations. Whole-cell currents were recorded with a solution containing 125 mM K^+ in the pipette and 5 mM, 70 mM or 125 mM K^+ in the extracellular bath. Membrane voltage was held at −50 mV and stepped to test potential values between −100 mV to 120-mV in 20-mV increments. Whole-cell currents were recorded after 4–5 min extracellular perfusion of a 290 mosmol/kg H_2O hypotonic solution: (*A*) in the presence of 5 mM K^+, (*B*) 70 mM K^+ and (*C*) 125 mM K^+ in the extracellular bath. (*D*) Average current voltage relationships measured 200 ms after onset of pulse, obtained from same cell at rest. Each value is the mean + SE of 5 cells obtained from 3 monolayers.

(10 μM) inhibited the swelling-activated K^+ current at + 80 mV by 75 ± 9% (n = 5), 62 ± 8% (n = 5) and 65 ± 6% (n = 5), respectively (Fig. 11*C*). In contrast, TEA (1 mM), charybdotoxine (10 nM) and 293B (10 μM) were inefficient in blocking outward currents since the % of inhibition at +80 mV were 4 ± 2% (n = 5) and 6 ± 3% (n = 5) respectively.

Discussion

Like cells from many other tissues, renal cells are capable of regulatory volume decrease (RVD) (Eveloff & Warnock, 1987; Strange, 1988; Knoblauch, Montrose & Murer, 1989; Tauc, Le Maout & Poujeol, 1990). When exposed to hypotonic media, the cells swell but subsequently return to their original volume. This RVD phenomenon is particularly important in the S1 and S2 part of the proximal tubules, as these segments exhibit numerous Na^+-solute cotransport systems that accumulate active osmolytes inside the cells. It is now well known that Cl^- and K^+ channels participate in the RVD response. However, the identity of these channels remains unclear.

Among the proteins that could be implicated in the RVD control, the KCNQ1/KCNE1 channels

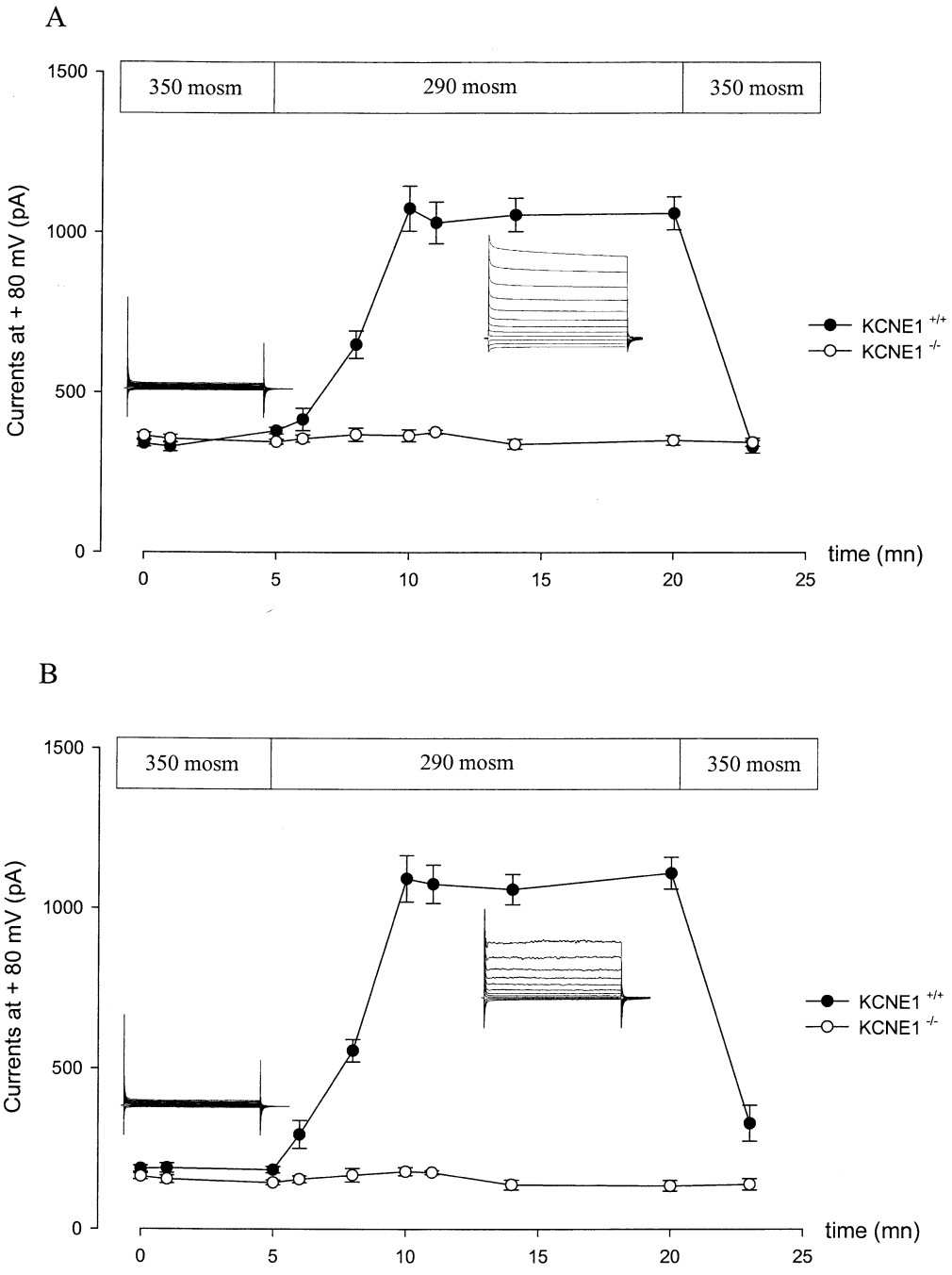

Fig. 9. Time course of K^+ and Cl^- currents induced by hypotonic shock in PCT cells from *kcne1* −/− and *kcne1* +/+ mice. (*A*) Time course of Cl^- currents at +80 mV measured 9 ms after onset of pulse as a function of bath osmolarity (20 cells from 5 monolayers). (*B*) Time course of K^+ currents at +80 mV measured 200 ms after onset of pulse as a function of bath osmolarity (12 cells from 4 monolayers).

represent possible candidates. The role of KCNE1 in cell volume regulation mechanisms was first suggested in the work of Wangeman et al. (1995) who demonstrated that hyposmotic challenge stimulated transepithelial K^+ secretion and activated KCNE1 channels in the apical membrane of vestibular dark cells. More recently Lock and Valverde have clearly suggested that the KCNE1 potassium channel subunit plays an important role in RVD in murine tracheal epithelial cells (Lock & Valverde, 2000). In the kidney, the experiment of Sugimoto et al. (l990) demonstrated the presence of KCNE1 along the proximal tubule. Moreover, the data of Chouabe et al. (l997) and Barhanin et al. (1996) indicate that the interaction between KCNQ1 and KCNE1 occurs in the kidney. However, in spite of these observations

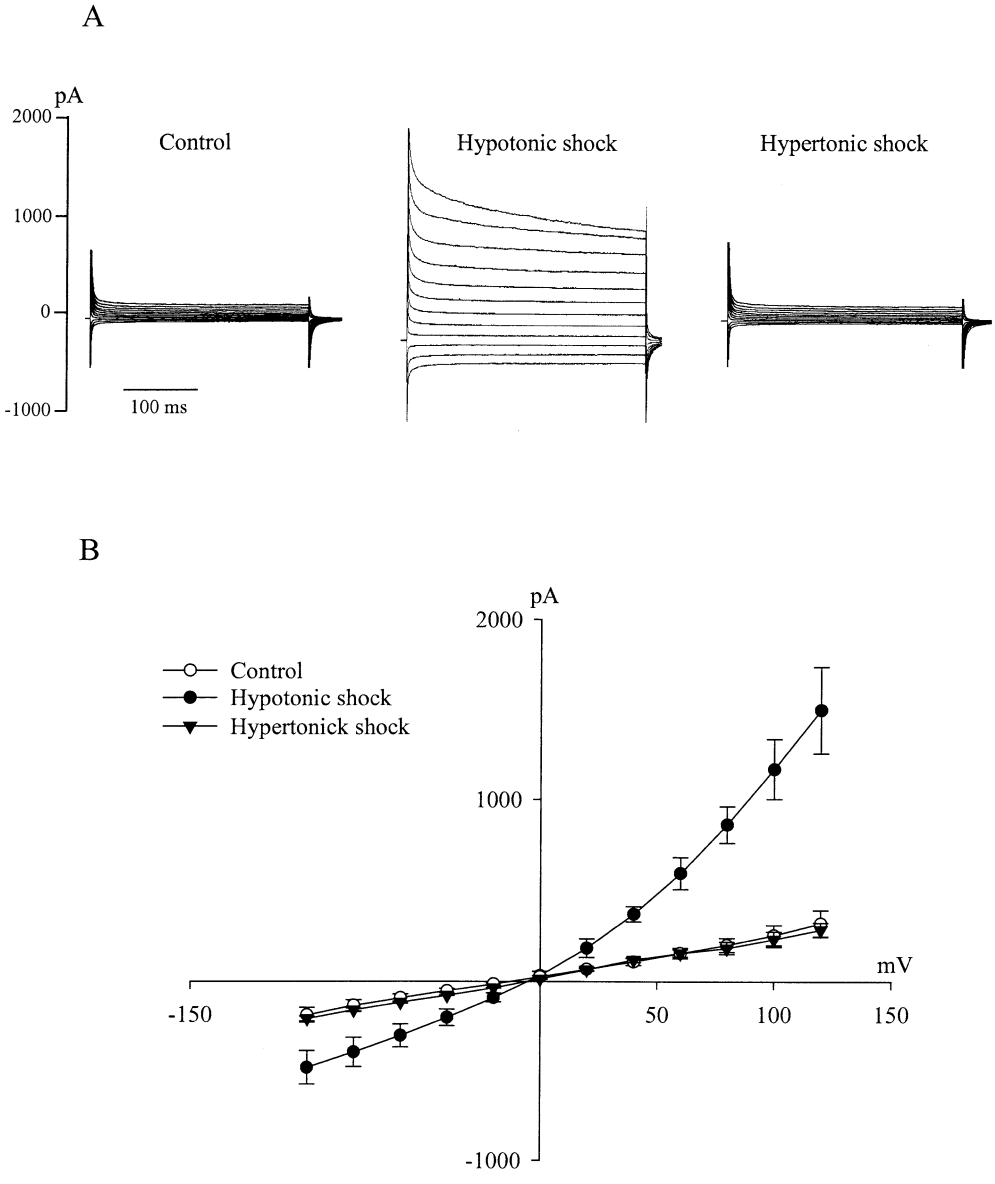

Fig. 10. Restoration of swelling-activated Cl^- currents by transitory tranfection of *plres-kcne1-CD8*. Transfected cells were visualized using anti-CD8 antibody-coated beads. Whole-cell currents were recorded with an isotonic solution containing 140 mM of NMDGCl in the pipette and extracellular bath. Membrane voltage was held at −50 mV and stepped to test potential values between −100 mV to 120 mV in 20-mV increments. (*A*) Whole-cell currents were recorded on cells labeled with anti-CD8-coated beads in control conditions, after 4–5 min extracellular perfusion of a 290 mosmol/kg H_2O hypotonic solution or after 3 min perfusion of a hypertonic solution. (*B*) Average current-voltage relationships measured 20 ms after onset of the pulse, obtained from the same cell at rest. Values are means ± SE of 4 cells obtained from 3 transfected monolayers.

nothing was known concerning the renal role of these proteins, notably in RVD control. To elucidate the role of KCNE1 in the proximal tubule, we performed primary cultures of microdissected nephron segments isolated from kidney of wild-type (*kcne1* +/+) and of knock-out (*kcne1* −/−) mice.

Primary cultures of microdissected nephron segments provide a useful tool for studying ion channels and transporters. Over the past 10 years, we have used this technique to investigate the nature and role of several ion channels in several segments of rabbit kidney such as proximal convoluted tubule (le Maout et al., 1990; Rubera et al., 1998), cortical ascending limb (Merot et al., 1991) and distal bright convoluted tubule (Merot et al., 1989; Poncet et al., 1994; Bidet et al., 1996; Tauc, Bidet & Poujeol, 1996; Rubera et al., 1997b) in primary cultures.

As expected, in PCT cells from control mice, swelling induced by exposure to hyposmotic solution was followed by RVD. This RVD process was strongly impaired by NPPB, confirming the implication of a Cl^--conductive pathway. Moreover, the

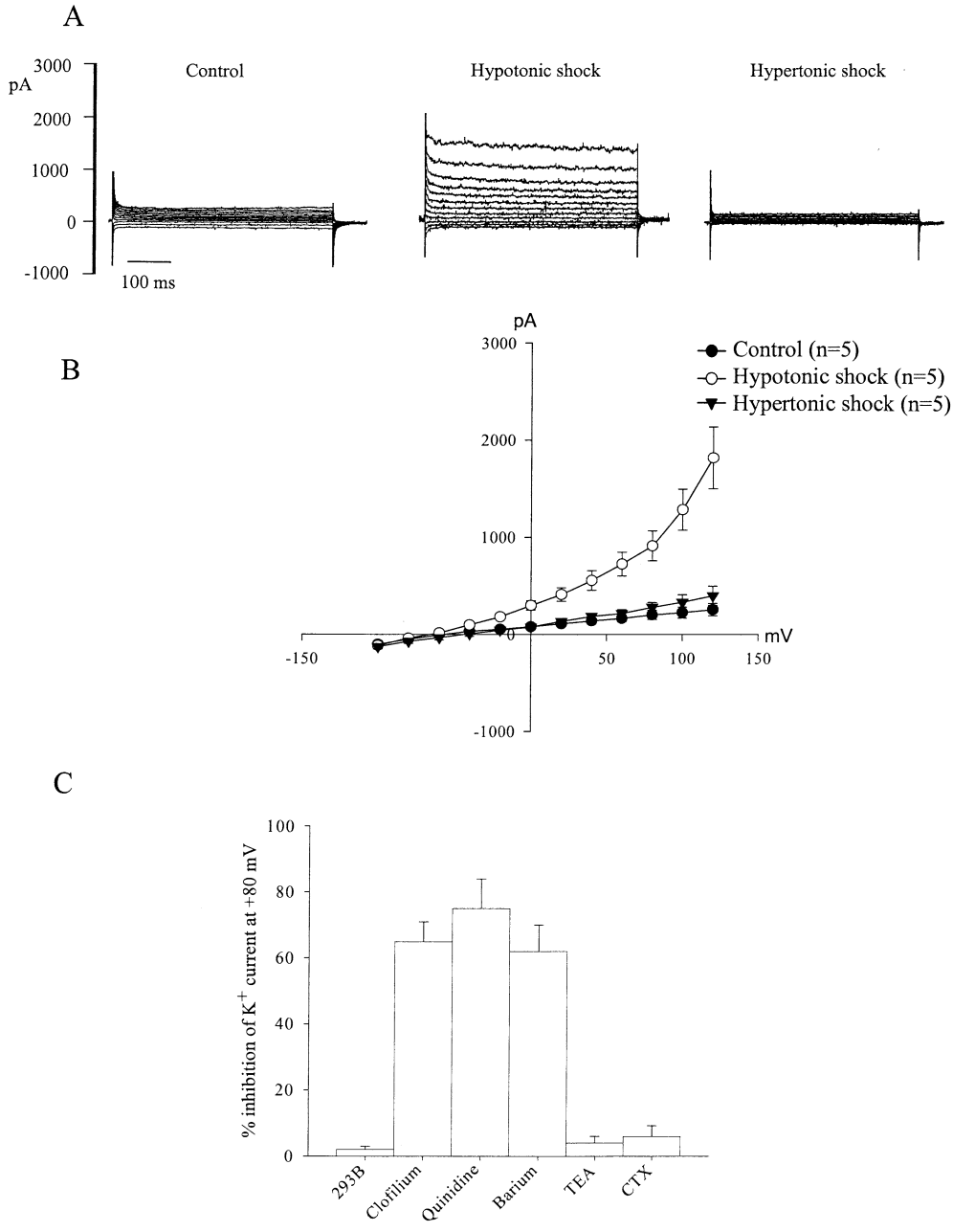

Fig. 11. Restoration of swelling-activated K^+ currents by transitory tranfection of plres-*kcne1-CD8*. Transfected cells were visualized using anti-CD8 antibody-coated beads. Whole-cell currents were recorded with an isotonic solution containing 125 mM K^+ in the pipette and 135 mM Na^+ in the extracellular bath. Membrane potential was held at −50 mV and stepped to test potential values between −100 and +120 mV in 20-mV increments. (*A*) Whole-cell currents were recorded in cells labeled with anti-CD8-coated beads in control conditions, after 4–5 min extracellular perfusion of a 290 mosmol/kg H_2O hypotonic solution or after 3 min perfusion of a hypertonic solution. (*B*) Average current-voltage relationships measured 200 ms after onset of pulse, obtained from the same cell at rest (*A*). Values are means ± SE of 5 cells obtained from 3 transfected monolayers. (*C*) Percentage of inhibition obtained with different K^+ channel inhibitors on swelling-activated K^+ currents on PCT cells from *kcne1* +/+ mice. Values are measured 200 ms after the onset of the pulse at + 80 mV. Each value is the mean ± SE of 5 cells obtained from 3 monolayers.

observation that quinidine completely blocked RVD indicates the involvement of K^+ conductance. It is now generally accepted that RVD is mediated by KCl loss via K^+ and Cl^- channels. In the present study, analysis of Cl^- and K^+ conductances by the whole-cell-clamp technique clearly showed that both conductances are increased by an osmotic shock in proximal cells from *kcne1* +/+ mice. The swelling-sensitive Cl^- conductance shares similar biophysical and pharmacological features with that described in

primary cultures of rabbit PCT cells (Rubera et al., 1998) and in other epithelial cells (Frizzell & Morris, 1994). Concerning the nature of the swelling-activated K^+ currents, the data in the literature are more puzzling since cell swelling could induce different types of K^+ channels (Wangemann et al., 1995; Lock & Valverde, 2000; Hoffmann & Hougaard, 2001; Niemeyer et al., 2001). In the present study, the insensitivity of the swelling-induced K^+ conductance to charybdotoxin indicates that the channel does not belong to the Ca^{2+}-activated maxi-K^+ channels. This channel, therefore, differs from the swelling-induced K^+ channel that was previously recorded in primary cultures of rabbit DCTb (Rubera et al., 1997b). In mouse PCT in primary culture, the swelling-induced K^+ conductance shared some properties with the K^+ currents activated by cell swelling that have been reported in gall bladder epithelium (Vanoye & Reuss, 1999) and in Ehrlich ascites tumor cells (Hoffmann, 2000). Notably, it is insensitive to TEA but is strongly blocked by barium, quinidine and clofilium. Moreover, the fact that this K^+ was recorded in the presence of high EGTA concentration in the pipette solution indicated that it is probably not affected by intracellular calcium concentration. According to Hoffmann (Hoffmann & Hougaard, 2001), this pharmacological profile is consistent with TASK channels. However, further experiments are required to definitively characterize the nature of the swelling-induced K^+ channels in PCT.

RVD was also examined in cultured proximal cells from *kcne1* −/− mice. Interestingly, these cells exhibited a defective volume regulation following a hyposmotic shock. This observation confirms results in the literature (Lock & Valverde, 2000) and indicates that KCNE1 could play a role in the RVD in proximal tubule. Obviously, this defective RVD is due to the fact that the hypotonic shock is completely inefficient in increasing K^+ and Cl^- conductances. A role for KCNQ1/KCNE1 channels in RVD has already been suggested in epithelial cells (Shiga & Wangemann, 1995; Wangemann et al., 1995; Lock & Valverde, 2000). In the present study, RT-PCR and Southern blot experiments clearly identified transcripts encoding the KCNQ1 and KCNE1 sequence in cultured PCT cells from *kcne1* +/+ mice. In these cells, the swelling-activated K^+ currents were insensitive to the chromanol derivative 293B (a strong blocker of KCNQ1 channels), indicating that KCNQ1 is not implicated in this conductance. Therefore, it appears that KCNQ1 is probably not the α-subunit for KCNE1 responsible for the volume-activated K^+ conductance in the proximal tubule. This conclusion is at variance with that reached by Lock and Valverde (2000) who proposed that the KCNQ1/KCNE1 complex is implicated in the maintenance of K^+ secretion linked to RVD in murine airway cells. As discussed above, the K^+ channel that is activated by cell swelling in PCT cells from *kcne1* +/+ mice could belong to the TASK family. The fact that this channel has never been recorded in PCT cells from *kcne1* −/− mice indicates that the null mutation of KCNE1 could lead to inactivation of this "TASK" channel.

Surprisingly, null mutation of the *kcne1* gene also strongly impaired the swelling-activated Cl^- currents. Before the demonstration by Barhanin et al. (1996) that KCNQ1 associates with KCNE1 to form a K^+ channel, Attali et al. (1993) had demonstrated that the KCNE1 protein acts as an activator of both K^+ and Cl^- channels. In particular, they showed that the Cl^- channel induced by KCNE1 transfection in *Xenopus* oocytes was potently inhibited by DIDS, whereas the K^+channel was not modified by the stilbene derivative. More recently several authors have reported a possible cAMP activation of a K^+ conductance in parallel with the stimulation of a Cl^- conductance in *cftr*-transfected cells (Mall et al., 2000). However, the K^+ conductance implicated could be the KCNQ1 channel (Bleich & Warth, 2000; Boucherot, Schreiber & Kunzelmann, 2001) and the intervention of KCNEl in the regulation of these currents remains controversial (Boucherot et al., 2001). Finally, more recent data provide evidence that KCNQ1 forming cAMP-activated K^+ channel associated with the KCNE3 subunit rather that KCNEl. We have previously demonstrated that the swelling-activated K^+ and Cl^- channels were not sensitive to cAMP (Rubera et al., 1997a; Rubera et al., 1997b). Furthermore, the present study indicates that KCNQ1 is not implicated in these swelling conductances. It is therefore very probable that in proximal cells KCNEl could interact with another type of subunit to control the Cl^- and K^+ channels implicated in the RVD phenomenon. As described by Greenwood et al. (2002) the KCNMB1 subunit (a β-subunit of BK channels) modulates the Ca^{2+}-activated Cl^- channel. In our case, KCNE1 could modulate the Ca^{2+} sensitivity of swelling-activated Cl^- currents. The nature of the interaction between KCNEl, Cl^- and K^+ channels remains completely unknown. Indeed, K^+ channels are required to drive Cl^- secretion in epithelia (Greger & Schlatter, 1984) and it could be that during RVD the exit of K^+ is the driving force for Cl^- efflux. However in the present study, the impairment of RVD in *kcne1* −/− cells is not only the consequence of the suppression of the driving force for Cl^- efflux due to the disappearance (or inactivation) of a swelling-sensitive K^+ channel but it is obviously linked to the absence (or inactivation) of the swelling-activated Cl^- channel itself. In other words, both types of channels are altered in cultured proximal cells from *kcne1* −/− mice. Such an alteration is unambiguously induced by the loss of KCNE1 protein since the transfection of *kcne1* −/− proximal cells with a cDNA encoding KCNE1

restored the activity of swelling-activated K^+ and Cl^- channels. Thus our results raise the question of the mechanisms by which KCNE1 could participate in the regulation of two different channels.

Finally the question arises to know whether the lack of volume-activated currents induced by the null mutation of KCNE1 is specific of kidney cells. Examining the expression of KCNE1 mRNA in mouse epithelia indicates that KCNE1 is abundant in the kidney, the stomach and the exocrine pancreas but is not expressed in the small intestine, the lung, the liver and the thymus (Demolombe et al., 2001). Obviously these tissues activate K^+ and Cl^- conductance to mediate RVD in response to cell swelling (Arrazola et al., 1993; MacLeod & Hamilton, 1996; vom Dahl et al., 2001). It appears therefore that volume-activated channels are probably not always coupled to the presence of KCNE1 protein. In other words, even if volume-activated Cl^- channels are ubiquitously expressed, their regulation by KCNE1 could be tissue-specific. The kidney is a good example of this specificity since the relation between KCNE1 and swelling-activated K^+ and Cl^- conductance exists in the PCT only. By contrast, the DCT, which did not express KCNE1 (Sugimoto et al., 1990), exhibits functional RVD associated with the activation of swelling K^+ and Cl^- conductances (Rubera et al., 1997b).

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