Role of TASK2 Potassium Channels Regarding Volume Regulation in Primary Cultures of Mouse Proximal Tubules

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ABSTRACT Several papers reported the role of TASK2 channels in cell volume regulation and regulatory volume decrease (RVD). To check the possibility that the TASK2 channel modulates the RVD process in kidney, we performed primary cultures of proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) from wild-type and TASK2 knockout (KO) mice. In KO mice, the TASK2 coding sequence was in part replaced by the lac-Z gene. This allows for the precise localization of TASK2 in kidney sections using β-galactosidase staining. TASK2 was only localized in PCT cells. K^+ currents were analyzed by the whole-cell clamp technique with 125 mM K-gluconate in the pipette and 140 mM Na-gluconate in the bath. In PCT cells from wild-type mice, hypotonicity induced swelling-activated K^+ currents insensitive to 1 mM tetraethylammonium, 10 nM charybdotoxin, and 10 μM 293B, but blocked by 500 μM quinidine and 10 μM clofilium. These currents were increased in alkaline pH and decreased in acidic pH. In PCT cells from TASK2 KO, swelling-activated K^+ currents were completely impaired. In conclusion, the TASK2 channel is expressed in kidney proximal cells and could be the swelling-activated K^+ channel responsible for the cell volume regulation process during osmolyte absorptions in the proximal tubules.

KEY WORDS: regulatory volume decrease • potassium conductance • two-pore K(+) channels • kidney • KCNK5

INTRODUCTION

In the large family of potassium channels, two pore domain K^+ channels exhibited diverse molecular and functional properties (Lesage and Lazdunski, 2000). Among these channels, TASK2 is highly expressed in the kidney, suggesting that it could play an important role in renal K^+ transport (Reyes et al., 1998). When expressed in *Xenopus laevis* oocytes, COS cells, HEK 293, or Ehrlich cells, cDNA encoding TASK2 generated K^+ currents that are insensitive to conventional K^+ channel blockers such as TEA (Duprat et al., 1997), apamin, or charybdotoxin (CTX;* Hoffmann, 2000), and inhibited by clofilium (Niemeyer et al., 2001a) and quinidine (Reyes et al., 1998). The most noteworthy properties of these currents are their complete insensitivity to external or internal Ca^{2+} and their strong dependence on external pH variations in a physiological range (Duprat et al., 1997; Reyes et al., 1998). However, the exact function of TASK2 in the kidney remains unknown because TASK2 K^+ channels have not yet been recorded in this organ.

Several works provide evidence that TASK channels could be much involved in Ehrlich cell regulatory volume decrease (RVD; Hoffmann, 2000). In these cells, the swelling-activated K^+ conductance presented functional similarities with TASK2 currents. The recent work of Niemeyer et al. (2001a) provides further evidence that when expressed in HEK 293 cells, TASK2 is modulated by changes in osmotic variation. According to these authors, the osmosensitivity of TASK2 suggests that it could participate in volume regulation in epithelial tissues where this protein is predominantly expressed.

In the different segments of the nephron, the cells are submitted to osmotic shocks either by accumulation of active osmolytes inside their cytoplasm (proximal tubule) or by dilution of the tubular fluid (distal tubule). These cells are capable of RVD in response to such osmotic stress, but the molecular identity of the Cl^- and K^+ channels activated to achieve this regulation remains unclear. Therefore, TASK2 channels could be involved in K^+ efflux during RVD. In a previous work in proximal convoluted tubule (PCT) in the murine kidney, a decrease in tonicity activated a current that, shown apparent open channel Goldman-Hodgkin-Katz rectification, was insensitive to TEA, CTX, and 293B, but blocked by quinidine and low concentration of clofilium (Belfodil et al., 2003). This current was Ca^{2+}-insensitive, but highly sensitive to external pH. This pharmacology pattern of the swelling-

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*Abbreviations used in this paper: CCT, cortical collecting tubule; CTX, charybdotoxin; DCT, distal convoluted tubule; KO, knockout; NPPB, 5-nitro-2-(3-phenylpropylamino)-benzoic acid; PCT, proximal convoluted tubule; RVD, regulatory volume decrease; X-gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside.

activated K^+ channels suggested that K^+ channels implicated in the control of cell volume could belong to TASK2 family (Belfodil et al., 2003). To verify this hypothesis, we developed primary cultures of renal tubules microdissected from kidney of either control (TASK2 +/+) or TASK2 knockout (KO) (TASK2 −/− mice). In these cells, K^+ and Cl^- channels activated by a hypotonic shock were measured using whole-cell clamp technique. The present results show that TASK2 is mainly expressed in proximal tubules in situ. Moreover, TASK2 K^+ channels are clearly functional in primary culture of proximal cells. Because KO of TASK2 completely abolished volume regulation in proximal cells, it is concluded that TASK2 K^+ channels are responsible for the K^+ efflux that occurs during RVD process in this segment.

MATERIALS AND METHODS

Animals

Dr. W.C. Skarnes (University of California, Berkeley, Berkeley, CA) provided the genetically modified mice used in this work. KCNK5 KO mice were generated by gene-trap insertion in mouse embryonic stem (ES) cells. General gene-trap strategy was described previously (Skarnes, 2000; Leighton et al., 2001; Mitchell et al., 2001). Heterozygous males carrying the disrupted KCNK5 gene were produced as described previously (Gerstin et al., 2003).

Mice were backcrossed to C57BL/6J mice for five generations. All the animals used in this work were of the N5F1 or N5F2 generation. Mice genotype was assessed by quantitative dot blot analysis on genomic DNA from tail biopsies using a neomycin probe according to the method described previously (Skarnes, 2000).

Gene-trap vector insertion was located into KCNK5 mRNA using RT-PCR. Sense primer was designed to m*Task-2* cDNA (5′-TCACCTCGGCCATCATATTC-3′; GenBank/EMBL/DDBL accession no. AF259395) and antisense primer (5′-AGTAGACTTCTGCACAGACACC-3′) was designed to the CD4 transmembrane domain sequence located in the 5′ region of the gene-trap vector. Sequence analysis of the PCR product demonstrated insertion of the trap vector at position 188 of the m*Task-2* cDNA, corresponding to the exon1–2 boundary.

Primary Cell Cultures

All experiments were performed in accordance with the guide for the care and use of laboratory animals (1996; National Academy of Sciences, Washington, DC), with the guidelines of the French Agricultural Office, and in compliance with the legislation governing animal studies.

Proximal, distal, and collecting tubules were microdissected under sterile conditions. Kidneys were perfused with Hank's solution (GIBCO BRL) containing 700 kU/liter collagenase (Worthington), and were then cut into small pyramids that were incubated for 1 h at room temperature in the perfusion buffer containing 160 kU/liter collagenase, 1% NuSerum, and 1 mM $CaCl_2$. They were continuously aerated. Pyramids were then rinsed thoroughly in the same buffer devoid of collagenase. 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 culture medium composed of equal quantities of DMEM and Ham's F12 medium (GIBCO BRL) containing 15 mM $NaHCO_3$, 20 mM HEPES, pH 7.4, 1% serum, 2 mM glutamine, 5 mg/liter insulin, 50 nM dexamethasone, 10 μg/liter epidermal growth factor, 5 mg/liter transferrin, 30 nM sodium selenite, and 10 nM triiodothyronine. Cultures were maintained at 37°C in a 5% CO_2/95% air water-saturated atmosphere. The medium was removed 4 d after seeding and then every 2 d.

Electrophysiological Studies

Whole-cell currents were recorded from 6 to 20-d-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; Propper Manufacturing Inc.) using a two-stage vertical puller (PP 83; Narishige) and filled with appropriate solutions (Table I). Osmolarity of bath solutions was adjusted to 350 mosm/kg with mannitol, and pH for both solutions was maintained at 7.4. Cells were observed using an inverted microscope with a stage equipped with a water robot micromanipulator (WR 89; Narishige). The patch pipette was connected via an Ag/AgCl wire to the headstage of a patch amplifier (model RK 400; 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 for by using settings available on the RK 400 amplifier. The series resistances were estimated by the equation $R_s = \tau/C_m$, where τ is the time constant of the exponential relaxation. The series resistances (R_s) of 100 experiments averaging 8.7 ± 0.9 MΩ were compensated, but experiments in which the series resistance was >20 MΩ were discarded. Cell membrane potentials (V_m) were measured at zero membrane current in the I_0 mode of the amplifier (current clamp to zero current). 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 Digidata 1200

TABLE I

Composition of Solutions Used in Whole-Cell Clamp Experiments

			Pipette	
	NMDGCl	Na-gluconate	NMDGCl	K-gluconate
Na^+	-	135	-	-
K^+	-	5	-	125
Ca^{2+}	1	1	-	-
$NMDG^+$	140	-	140	-
Cl^-	142	2	140	20
Gluconate	-	140	-	105
Glucose	5	5	-	-
Mg-ATP	-	-	5	5
EGTA	-	-	5	5
HEPES	10	10	10	10
Mannitol	60	60	0	0
Osmolarity (mosmol/kg H_2O)	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 and were made hyperosmotic by addition of mannitol.

interface (Axon instruments, Inc.). PCLAMP software (version 5.51 and 6.0; Axon Instruments, Inc.) 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's 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.

5-Bromo-4-chloro-3-indolyl β-D-galactoside (X-gal) Staining

For β-galactosidase histochemistry, kidneys from wild-type and KO mouse were removed and fixed in 4% paraformaldehyde in PBS overnight. After rinsing overnight in PBS, 40-μm sections were obtained using a vibratome. Floating sections were stained overnight at 37°C in the following mixture: 0.1 M phosphate buffer, pH 7.3, X-gal 1 mg/ml stock solution at 25 mg/ml in dimethylformamide, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, and 2 mM $MgCl_2$.

Cell Volume Measurements

The relative cell volume was monitored by image analysis with Fura2 as the fluorescent volume indicator as previously reported (Rubera et al., 1997). 6–20-d-old cell monolayers grown on a Petri dish were loaded with a solution of 2 μM Fura2 containing 0.01% pluronic acid for 20 min at 37°C, and were then washed with an NaCl solution. The fluorescence was monitored with a 360-nm excitation wavelength. At 360 nm, the variations of the signal emitted by the probe are directly proportional to the variations of cell volume. In a typical experiment, the cells were first perfused with an isotonic NaCl solution containing (mM): 110 NaCl, 5 KCl, 1 $CaCl_2$, 90 mannitol, and 10 HEPES, pH 7.4 (osmotic pressure [Posm] = 300 mosmol/kg H_2O) at 30 ml/min, and images were averaged eight times and recorded every 5 s for 15 min. Once the fluorescence was stabilized, a hypotonic shock was induced by perfusing the NaCl solution without mannitol (Posm = 200 mosmol/kg H_2O). The estimation of the relative change in cell volume from the fluorescent signal was made, assuming that a 30% decrease in 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 analysis of 10–20 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 an inverted microscope (model ICM-405; Carl Zeiss MicroImaging, Inc.) and a 40× objective (Carl Zeiss MicroImaging, Inc.), which was used for epifluorescent measurement with a 75-W xenon lamp. The excitation beam was filtered through a narrow-band filter centered at 360 nm mounted in a motorized wheel (Lambda 10–2; Sutter Instrument Co.) and equipped with a shutter to control the exposure times. The incident and the emitted fluorescence radiation were separated through a chromatic beam splitter (Carl Zeiss MicroImaging, Inc.). 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). 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 gray levels, ranging from 0 (for black) to 255 (for white). Image acquisition and analysis were performed with the 2.0 version of AIW software (Axon Instruments). The final calculations were made using Excel software (Microsoft).

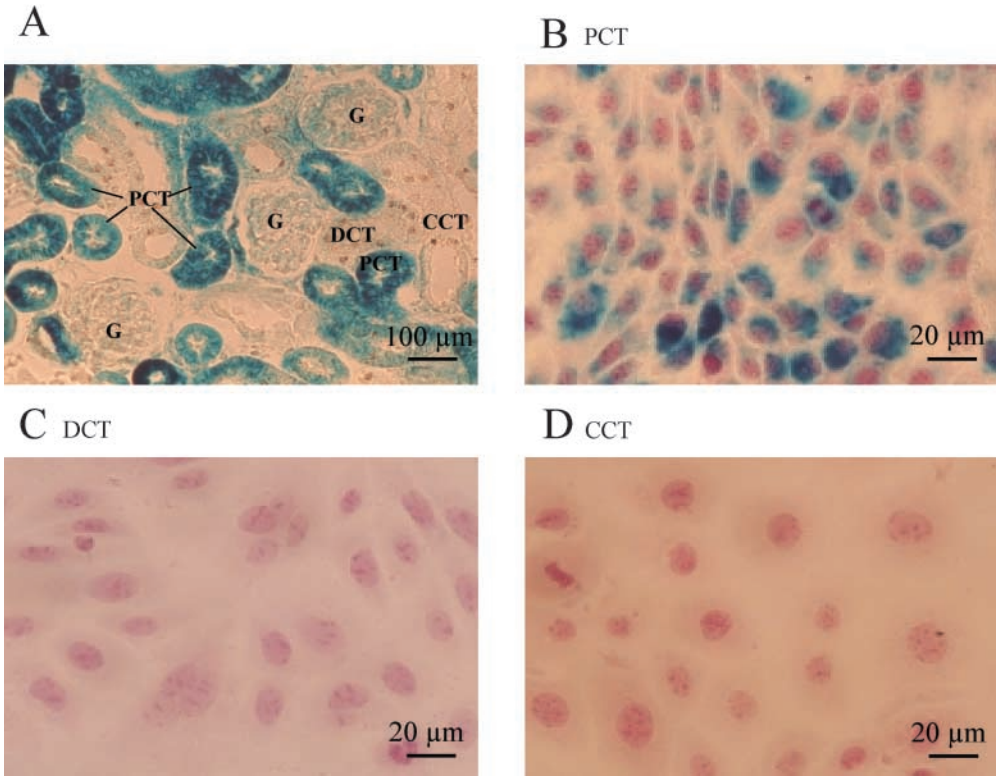

FIGURE 1. Lac Z expression in renal cells. Kidney sections or primary cultures from TASK2 −/− mice were incubated with X-gal overnight at 37°C. (A) X-gal staining in cortical kidney sections (G, glomerulus; PCT, proximal convoluted tubules; DCT, distal convoluted tubules; CCT, cortical collecting tubules); (B) in primary cultures of proximal cells; (C) in primary cultures of distal cells; and (D) in primary culture of collecting cells.

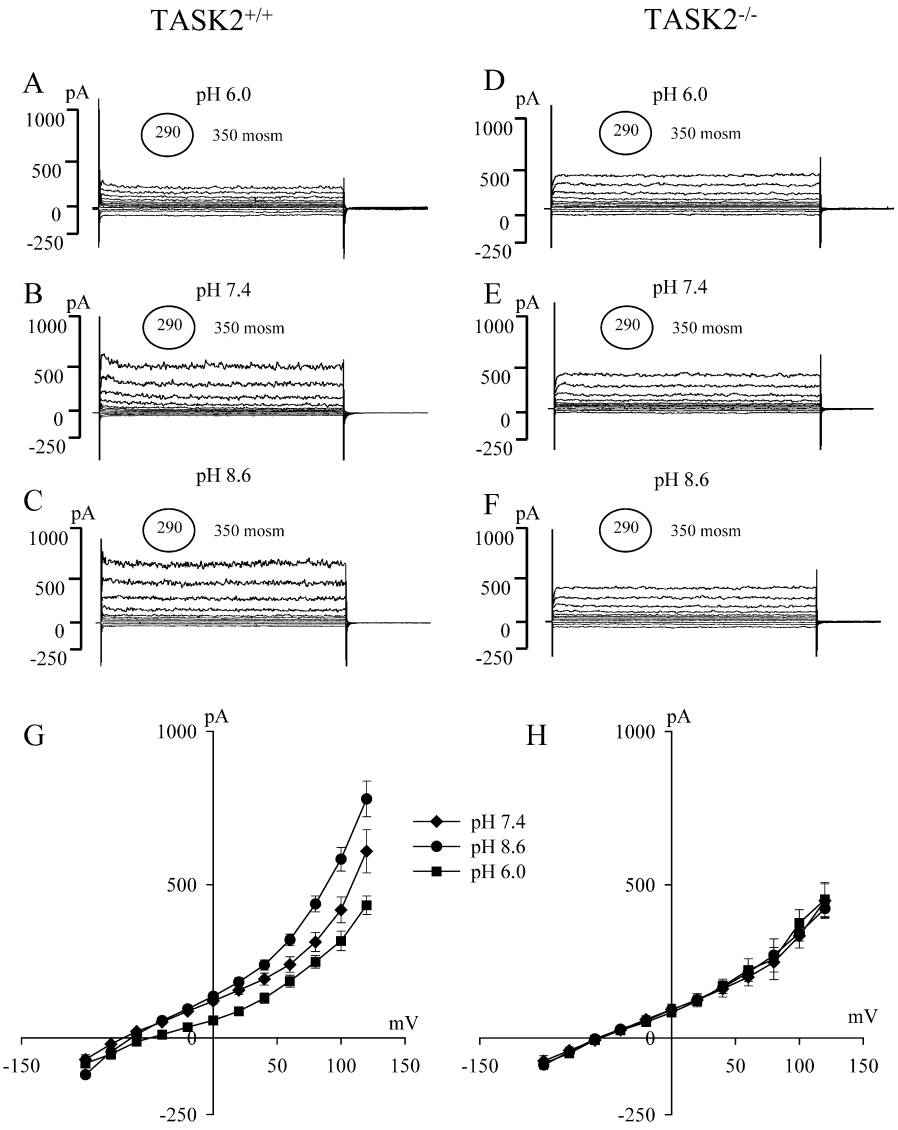

FIGURE 2. Effect of external pH variations on K^+ currents in cultured PCT cells from *TASK2* +/+ and *TASK2* −/− mice. Under each experimental condition, the circled values represent the osmotic pressure in the pipette, and the values outside the circle represent the osmotic pressure in the extracellular bath solutions. Membrane voltage was held at −50 mV and stepped to test potential values between −100 and +120 mV in 20-mV increments. Whole-cell currents were recorded with a 350-mosmol/kg H_2O solution in the extracellular bath containing 5 mM of K^+, and a 290-mosmol/kg H_2O solution in the pipette containing 125 mM of K^+. (A and D) Whole-cell currents recorded at a pH of 6.0. (B and E) Whole-cell currents recorded at a pH of 7.4. (C and F) Whole-cell currents recorded at a pH of 8.6. (G and H) Average current-voltage relationships measured at 200 ms after onset of pulse, obtained from same cells at rest. Values are means ± SEM of (*n*) different cells from six different mice.

Calibration

We used the methods described by Tauc et al. (1990) using BCECF, and improved more recently by Raat et al. (1996) using Fura2. After loading with the fluorescent probe in the culture medium, the cells were perfused with a solution adjusted to various osmolarities (150–400 mosm) omitting mannitol. For each osmolarity, two images were stored, averaged, and subsequently corrected for fading after background subtraction. The mean fluorescence (360 nm) of five areas was plotted against the inverse of the osmotic pressure (in mosmol). Data showed that when the cells were exposed to a hypoosmotic solution, fluorescence decreased linearly with the osmotic pressure according to Boyle's law. To check that cells in cultures behave as osmometers in a reversible manner, experiments were performed in which the cultures were perfused successively and randomly with solutions of various osmolarities (ranging from 200 to 300 mosm). The fluorescent signal was related to the osmotic pressure in a reversible way. In all calibration experiments, images were recorded 1–2 min after the beginning of the perfusion, at which time the swelling in hypotonic solutions reached was maximum value. These methods measure the variations of the relative volume as a function of the osmotic pressure of the perfusion medium (Tauc et al., 1990).

Chemicals

5-Nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB; Calbiochem) was prepared at 100 mM in DMSO and used at 0.1 mM in final solutions. Clofilium tosylate, TEA, CTX, quinidine, and apamin were obtained from Sigma Aldrich. Fura2 a.m. (Molecular Probes, Inc.) was dissolved at 3 mM in DMSO and added to the loading solution at a final concentration of 2 μM plus 0.01% pluronic acid. 293B was prepared at 10 mM in DMSO and used at 10 μM in final solutions. Clofilium was prepared at 10 mM in 50% DMSO, 50% water, and was used at a final concentration of 10 μM. 293B was gift from Dr. Barhanin (AVENTIS Pharma SA, Paris, France.)

RESULTS

Expression of TASK2 in Renal Cells

To check whether renal tissue and cultured cells monolayers expressed TASK2 protein, we used *TASK2* −/− β-gal mice in which the gene encoding TASK2 was replaced by the gene encoding β-galactosidase. In tissue slices or primary cultures from these mice, the positive

blue staining after X-gal reaction indicated the presence of β-galactosidase instead of TASK2. Fig. 1 A illustrates the-galactosidase/X-gal reaction performed on kidney cortex slices from *TASK2* −/− β-gal mice. In these slices, the blue staining was found abundantly in proximal tubules. β-galactosidase histochemistry was also performed on primary cultures of PCT, distal convoluted tubule (DCT), and cortical collecting tubule (CCT) cells from *TASK2* −/− β-gal mice. A positive blue staining was only observed in PCT cells and not in DCT or CCT cells (Fig. 1, B–D). From these experiments, it could be concluded that in mouse kidney cortex, TASK2 protein is probably expressed in proximal tubule.

Effect of External pH Variations on K^+ Currents in Cultured PCT Cells from TASK2 +/+ and TASK2 −/− Mice

Fig. 2 (left) shows the currents recorded using an isotonic (290 mosmol/kg H_2O) solution in the pipette and a hypertonic (350 mosmol/kg H_2O) solution in the bath in PCT cells of TASK2 +/+. With high K^+ (125 mM) in the pipette and low K^+ (5 mM) in the bath solutions, the currents recorded at an external pH (pH_e) of 7.4 were outward rectifying with a maximal slope conductance of 9.6 ± 1.0 nS and a reversal potential of −73.4 ± 2.6 mV (n = 20). Increasing pH_e to 8.6 enhanced the currents, whereas decreasing pH_e to 6.0 decreased the currents. Thus, the maximal slope conductances were 10.4 ± 1.3 nS (n = 34) and 5.8 ± 1.1 nS (n = 8) at a pH_e of 8.6 and 6.0, respectively. These values were significantly different (unpaired t test; $P < 0.01$). The variations of pH_e did not significantly affect the reversal potentials (E_{rev} at pH 8.6 = −71.2 ± 2.4 mV, n = 34; E_{rev} at pH 6.0 = −63.6 ± 6.0 mV, n = 8, NS). Under these experimental conditions, the reversal potentials were close to the Nernst potential for K^+ ions, indicating that the currents were mainly carried by K^+. However, to strengthen this observation, experiments were performed replacing external bath Na^+ (135 mM) by K^+. This maneuver clearly shifts the reversal potentials close to 0 mV and did not modify the pH dependence characteristics of the currents (unpublished data).

K^+ conductances were also recorded in PCT cells from *TASK2* −/− mice. The results are reported in Fig. 2 (right). In the presence of high K^+ in the pipette solution and at a pH_e of 7.4, the stimulation protocol elicited small currents that reversed at E_m = −68 0.0 ± 4.0 mV (n = 16). These currents were lower than those measured in *TASK2* +/+ cells under identical experimental conditions (maximal slope conductance = 5.6 ± 1.3 nS, n = 16). In contrast to that observed in *TASK2* +/+ cells, the currents remained insensitive to pH_e variations (Fig. 2 H). These results suggest that TASK2 K^+ channels are implicated in the pH-sensitive K^+ conductance in PCT cells.

To further characterize the pH-sensitive K^+ current in PCT cells from *TASK2* +/+ mice, the effect of different K^+ channel blockers was tested on K^+ conductance measured at a pH_e of 8.6. As shown in Fig. 3 A, 0.5 mM quinidine and 10 μM clofilium inhibited the K^+ currents at 100 mV by >60%. In contrast, 1 mM

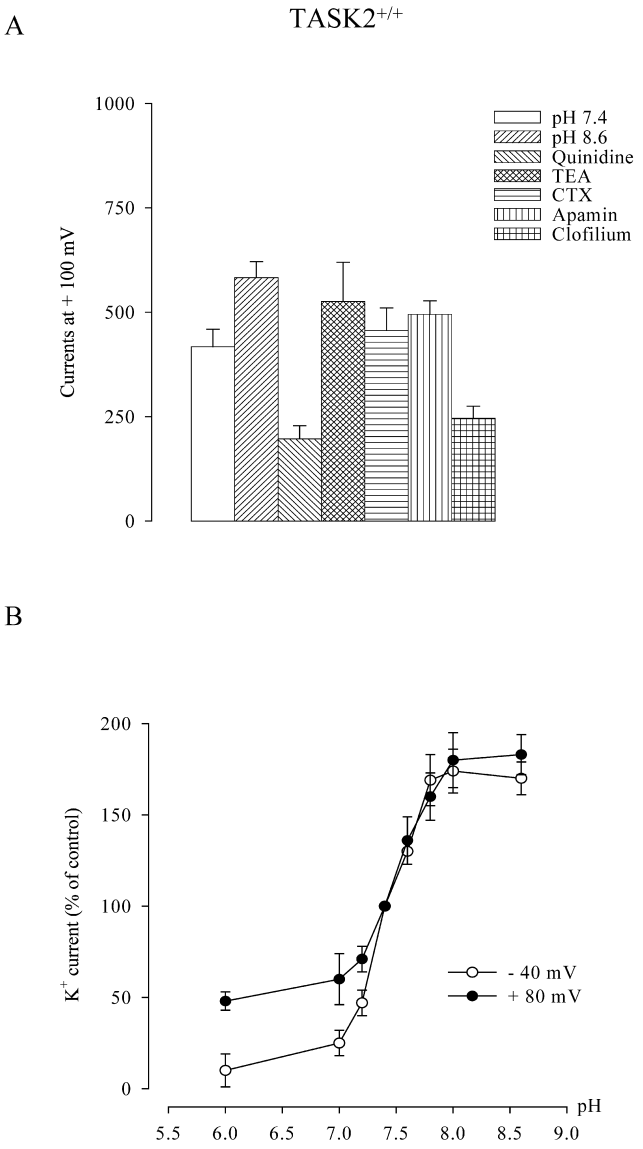

FIGURE 3. Effect of different K^+ channel inhibitors and relation between K^+ currents and pH on PCT cells from *TASK2* +/+ mice. Membrane voltage was held at −50 mV and stepped to test potential values between −100 and +120 mV in 20-mV increments. Whole-cell currents were recorded with solutions containing 125 mM of K^+ in the pipette and 5 mM of K^+ in the extracellular bath. (A) Histogram of current values at 100 mV in the presence of different K^+ channel effectors on K^+ currents in PCT cells from *TASK2* +/+ mice. Whole-cell currents were recorded 3 min after the perfusion of an extracellular solution containing 0.5 mM quinidine, 1 mM TEA, 10 nM CTX, 10 nM apamin, or 10 μM clofilium. Values are measured 200 ms after the onset of pulse at 100 mV. Each value is mean ± SEM of 15 cells obtained from five monolayers. (B) Relation between external pH and K^+ currents. Whole-cell currents were recorded 3 min after perfusion of an extracellular solution at different pH. Values are measured at 200 ms after the onset of pulse at −40 and +80 mV. Each value is mean ± SEM of eight cells obtained from four monolayers.

TEA, 10 nM CTX, or 10 nM apamin did not significantly modify the K^+ currents.

Fig. 3 B shows the strong pH dependence of the inward and outward K^+ currents. Varying the pH_e values between 6.0 and 8.6 indicated that the currents were very sensitive to extracellular pH within the physiological range.

K^+ Currents Induced by a Hypotonic Shock in Cultured PCT Cells from TASK2 +/+ and TASK2 −/− Mice

To study the effect of changes in osmotic pressure on the development of K^+ conductance, currents were induced by osmotic shock. In these experiments, whole-cell currents were recorded with Ca^{2+}-free pipette solutions containing 125 mM K^+ and maintained at an osmolarity of 290 mosmol/kg H_2O and a pH of 7.4. Fig. 4 A shows K^+ currents observed in cultured PCT from TASK2 +/+ mice with an extracellular solution osmolarity of 350 mosmol/kg H_2O and at a pH of 7.4. The voltage step protocol elicited small time-independent outwardly rectifying currents that had a reversal potential of −72.2 ± 4 mV and a maximal slope conductance of 3.3 ± 0.9 nS (n = 20).

The monolayers were then perfused with a 290 mosmol/kg H_2O solution. Fig. 4 B illustrates the development of the currents obtained 4–5 min after the onset of the hypotonic shock. These large, outwardly rectifying currents reversed at −73.1 ± 2.0 mV with a maximum slope conductance of 17.8 ± 1.3 nS (n = 20). When the cells were reexposed to the hyperosmotic

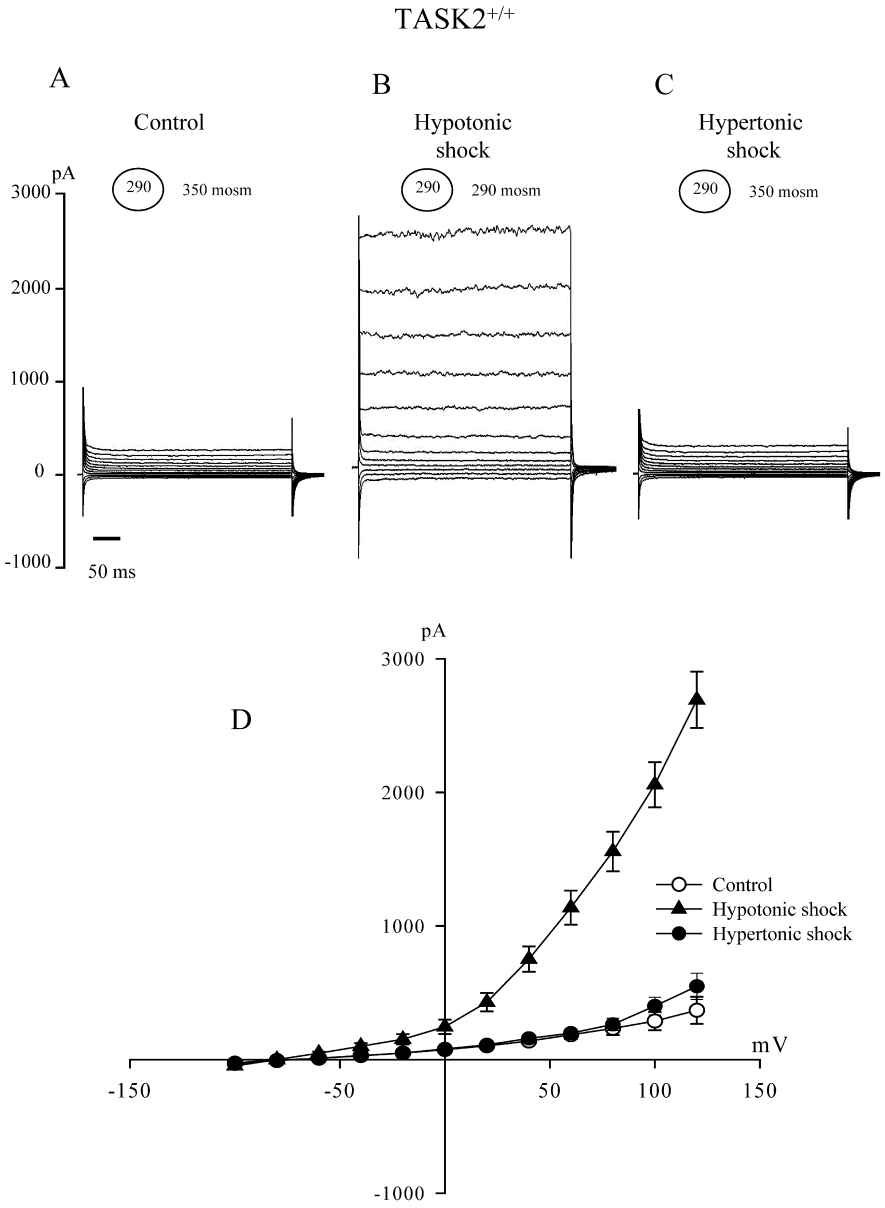

FIGURE 4. Characteristics of swelling-activated whole-cell K^+ currents in primary cultures of PCT cells from *TASK2* +/+ mice. Under each experimental condition, the circled values represent the osmotic pressure in the pipette, and the values outside the circle represent the osmotic pressure in the extracellular bath solutions. Whole-cell currents were recorded with solutions containing 125 mM of K^+ in the pipette and 5 mM of K^+ in extracellular bath. Membrane voltage was held at −50 mV and stepped to test potential values between −100 and +120 mV in 20-mV increments. (A) Whole-cell currents recorded in control conditions (350 mosmol/kg H_2O in extracellular bath) after the mechanical rupture of membrane. (B) Whole-cell currents recorded 4–5 min after extracellular perfusion of a 290-mosmol/kg H_2O hypotonic solution. (C) Effect of a hypertonic solution (350 mosmol/kg H_2O) on swelling-induced whole-cell K^+ currents. (D) Average current-voltage relationships measured 200 ms after onset of pulse, obtained from same cell at rest. Values are means ± SEM of 20 different cells from five different monolayers.

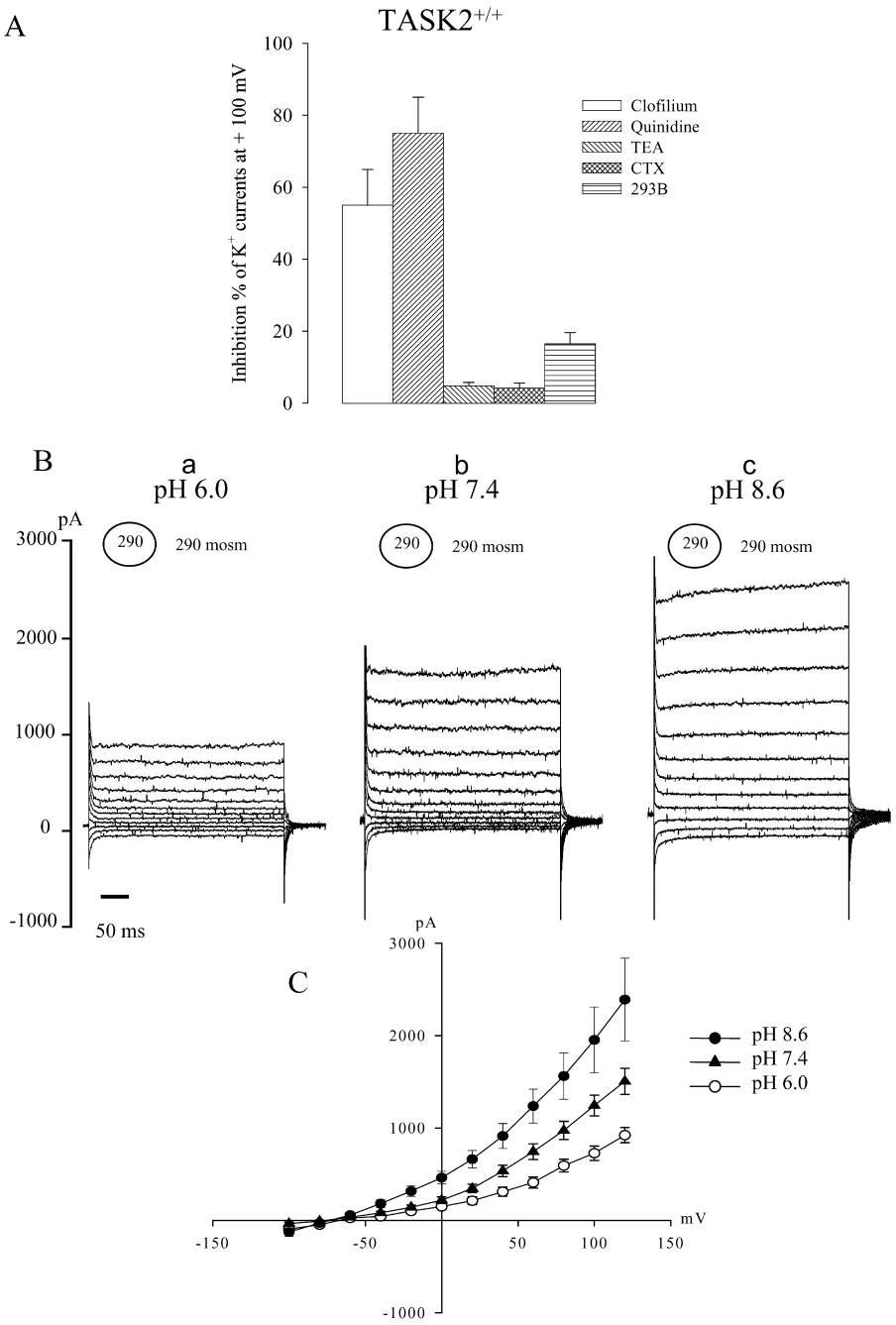

FIGURE 5. (A) Histogram of percent inhibition of different K^+ channel inhibitors on swelling-activated K^+ currents recorded in PCT cells from *TASK2* +/+ mice. Membrane voltage was held at −50 mV and stepped to test potential values between −100 and +120 mV in 20-mV increments. Whole-cell currents were recorded 4–5 min after extracellular perfusion of a 290-mosmol/kg H_2O hypotonic solution in the presence of 10 μM clofilium, 0.5 mM quinidine, 1 mM TEA, 10 nM CTX, and 10 μM 293B. Values measured 200 ms after onset of pulse at 100 mV are converted to percent inhibition. Each value is mean ± SEM of (*n*) cells obtained from six monolayers. (B) Effect of extracellular pH on the development of swelling-activated K^+ currents in cultured PCT cells from *TASK2* +/+ mice. Under each experimental condition, the circled values represent the osmotic pressure in the pipette and the values outside the circle represent the osmotic pressure in the extracellular bath solutions. Membrane voltage was held at −50 mV and stepped to test potential values between −100 and +120 mV in 20-mV increments. (Ba, Bb, and Bc) Whole-cell currents were recorded 4–5 min after extracellular perfusion of a 30% hypotonic solution at pH 6.0, 7.4, and 8.6, respectively, in the presence of 5 mM EGTA and 5 mM Mg-ATP in the pipette solution. (C) Corresponding average current-voltage relationships measured 200 ms after onset of pulse, obtained from same cell at rest and during hypotonic stimulation at pH 6.0, 7.4, and 8.6. Values are means ± SEM of (*n*) cells obtained from five monolayers.

solution (350 mosmol/kg H_2O), the currents returned to the control level within 2–3 min (Fig. 4, C and D). To characterize more fully these swelling-activated K^+ currents, the effects of several inhibitors known to affect different K^+ channels were tested. The data are reported in Fig. 5 A. As reported in the previous paragraph for K^+ currents recorded in isotonic conditions, quinidine strongly inhibited, whereas TEA and CTX did not significantly modify, the swelling K^+ conductance. Additionally, 10 μM clofilium also blocked these conductances and 293 B remained without significant effect. This inhibitory profile indicated that the K^+ channels implicated in the increase of the K^+ conductance induced by hypotonicity belong to the TASK family. To confirm this observation, the effect of hypotonic shock was studied at different external pH values. As expected for TASK2 channels, the hypotonic-stimulated K^+ currents were significantly increased at a pH_e of 8.6 (maximal slope conductance 26.3 ± 2.9 nS, n = 6) and decreased at a pH_e of 6.0 (maximal slope conductance 6.6 ± 1 nS, n = 9) as compared with the currents measured at a pH_e of 7.4 (maximal slope conductance: 11.8 ± 2.9 nS, n = 15; Fig. 5, B and C).

The effect of hypotonicity was also studied in PCT cells from *TASK2* −/− mice. In 100% of the monolayers tested, the hypotonic shock was completely inefficient in increasing K^+ currents (Fig. 6). However, in these cells, the hypotonic shock enhanced Cl^- currents that were blocked by returning in a hypertonic solution (Fig. 7, B and C). In symmetrical Cl^- concentrations, these swelling-activated currents rectified in outward direction and reversed at −0.5 ± 1.5 mV (n = 4; Fig. 7 D). As they strongly resembled swelling-activated Cl^- currents previously found in wild-type mice (Rubera et al., 1998; Barriere et al., 2003), they were not analyzed further.

Influence of Hypotonic Shock on Relative Cell Volume in Cultured PCT Cells from TASK2 +/+ and TASK2 −/− Mice

In previous work (Belfodil et al., 2003), it was postulated that TASK2 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. 8 A. 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 *TASK2* +/+ mice. 2 min after the hypotonic shock, the relative cell volume reached 130.1 ± 2% (n = 4 monolayers) of the initial volume and returned to 105. 2 ± 1% (n = 4 monolayers) of the original volume within 4 min. In these cells, the efficient RVD process was completely blocked in the presence of 100 μM NPPB or 10 μM clofilium, indicating that Cl^- and K^+ channels were probably involved in this process. In contrast, the RVD phenomenon was completely impaired in cells originating from *TASK2* −/− mice; when perfused with the hypotonic solution, these cells never returned to their initial volume (Fig. 8 A).

The effects of extracellular Ca^{2+} on the RVD process were tested in PCT from TASK2 +/+ cells. Fig. 8 B shows that when hypotonic shock was performed in the absence of bath Ca^{2+}, the RVD was significantly impaired. The PCT cells exhibited a similar increase of relative cell volume after 2 min of hypotonic shock in the presence or absence of bath Ca^{2+}; respectively, 129.9 ± 1.2% (n = 4 monolayers) and 132.0 ± 1.0% (n = 4 monolayers). In the presence of extracellular Ca^{2+}, the cells returned to their initial volume within 6 min after the induction of hypotonic shock (98.1 ± 1.2%, n = 4 monolayers). In contrast, in the absence of external Ca^{2+}, the cells did not regain their original volume (122.1 ± 1.1%, n = 4 monolayers). The necessity of the presence of Ca^{2+} in the extracellular bath was tested on the swelling-activated currents during hypotonic shock. As shown in Fig. 9 A, the absence of extracellular Ca^{2+} completely impaired the swelling-activated Cl^- currents.

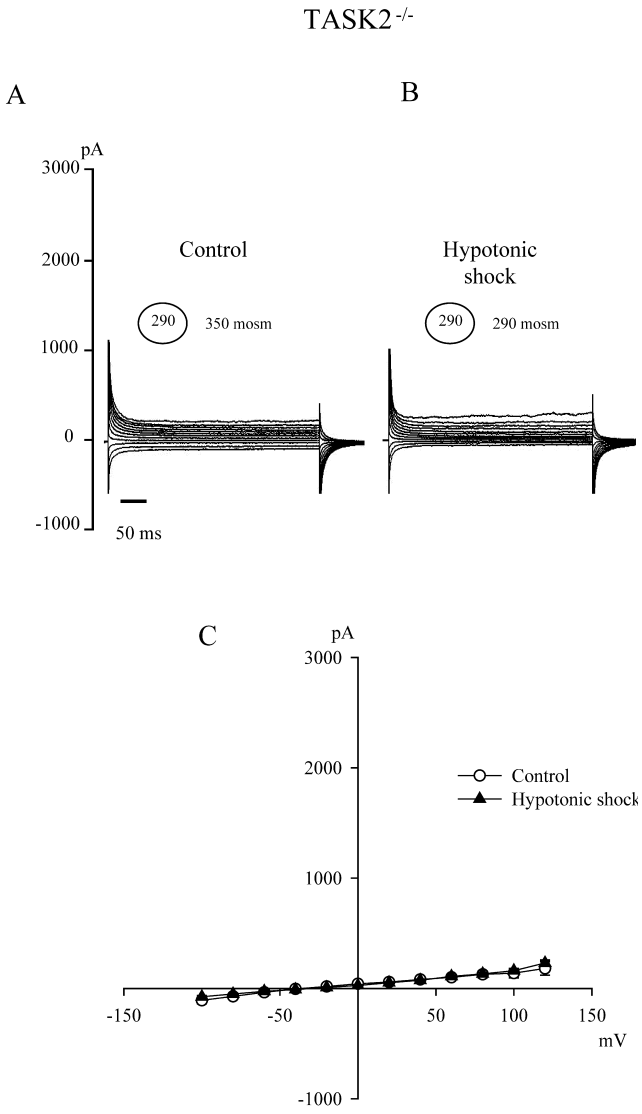

FIGURE 6. Characteristics of swelling-activated whole-cell K^+ currents in primary cultures of PCT cells from *TASK2* −/− mice. Under each experimental condition, the circled values represent the osmotic pressure in the pipette, and the values outside the circle represent the osmotic pressure in the extracellular bath solutions. Whole-cell currents were recorded in solutions 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 and +120 mV in 20-mV increments. (A) Whole-cell currents recorded in control conditions (350 mosmol/kg H_2O in the extracellular bath) after the mechanical rupture of membrane. (B) Whole-cell currents recorded 4–5 min after extracellular perfusion of a 290-mosmol/kg H_2O hypotonic solution. (C) Average current-voltage relationships measured at 200 ms after onset of pulse, obtained from same cell at rest. Values are means ± SEM of 20 cells from five different monolayers.

As concerns swelling-activated K^+ currents, they were not altered by the absence of Ca^{2+} (Fig. 9 B). Together, these results indicated that TASK2 currents are insensitive to external Ca^{2+}, whereas for swelling-activated Cl^- currents, Ca^{2+} was required for activation. As previously

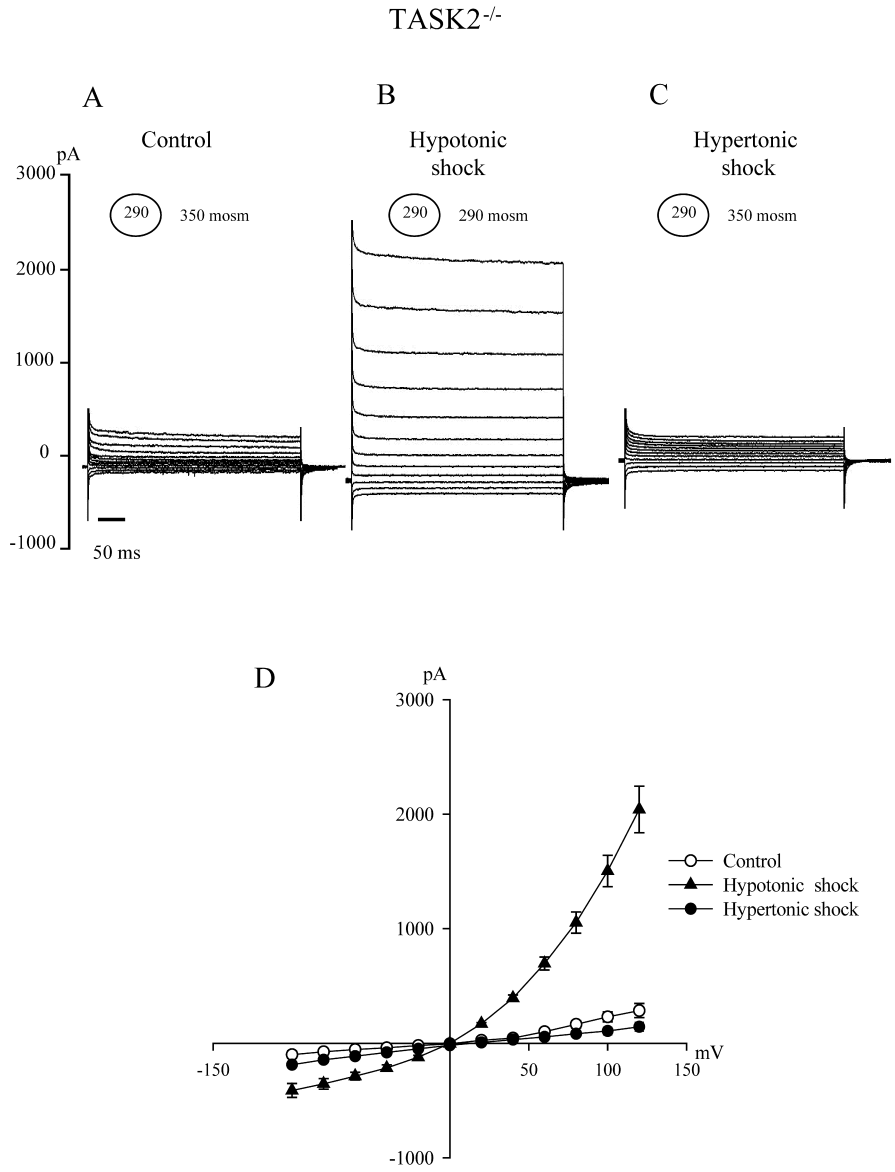

FIGURE 7. Characteristics of swelling-activated whole-cell Cl^- currents in primary cultures of PCT cells from *TASK2* −/− mice. Under each experimental condition, the circled values represent the osmotic pressure in the pipette, and the values outside the circle represent the osmotic pressure in the extracellular bath solutions. Whole-cell currents were recorded in solutions containing 140 mM NMDG-Cl in the pipette and in the extracellular bath. Membrane voltage was held at −50 mV and stepped to test potential values between −100 and +120 mV in 20-mV increments. (A) Whole-cell currents recorded in control conditions (350 mosmol/kg H_2O in the extracellular bath) after mechanical rupture of membrane. (B) Whole-cell currents recorded 4–5 min after extracellular perfusion of a 290-mosmol/kg H_2O hypotonic solution. (C) Effects of a hypertonic solution (350 mosmol/kg H_2O) on swelling-induced whole-cell Cl^- currents. (D) Average current-voltage relationships measured at 9 ms after onset of pulse. Each value is mean ± SEM of five cells obtained from three monolayers.

reported in rabbit kidney cells in primary cultures (Rubera et al., 2001), extracellular Ca^{2+} was required to induce the swelling-activated Cl^- currents (Fig. 9 A).

K^+ Currents Induced by Hypotonic Shock in Cultured DCT Cells from TASK2 +/+ and TASK2 −/− Mice

To prevent the development of Cl^- conductance, the effect of hypotonic swelling was tested in the presence of 0.1 mM NPPB in the bath solution. Control currents were recorded using an isotonic-free (290 mosmol/kg H_2O) Ca^{2+} (1 mM EGTA) solution in the pipette and a hypertonic (350 mosmol/kg H_2O) solution containing 1 mM Ca^{2+} in the bath. In DCT cells, the voltage-step protocol elicited small time-independent currents that changed linearly with the membrane voltage. The values of currents at 100 mV were 126 ± 16 pA (n = 20 cells from five different mice) in DCT cells. Because of their small amplitude, the nature of these currents was not analyzed further. To produce a hypotonic shock, the monolayers were then perfused continuously with a 290-mosm/kg H_2O solution. In 75% of *TASK2* +/+ cells, an increase in whole-cell current was observed in 3 min. In the two cell types, the currents reached a maximum in 4–5 min. Fig. 10 A (b) and Fig. 10 B (b) give the currents recorded in DCT cells from *TASK2* +/+ and *TASK2* −/− during hypotonic shock. In *TASK2* +/+ cells, the currents recorded in each cultured segment showed virtually no inactivation during the 400-ms pulse, and the channels involved in this conductance were activated at depolarized potentials. The slope conductances measured at 100 mV were 12–16 times the amplitude of those calculated at −60 mV. The reversal potentials were near to the equilibrium potential for K^+ ions in DCT segment (E_{rev} = −80 ± 4

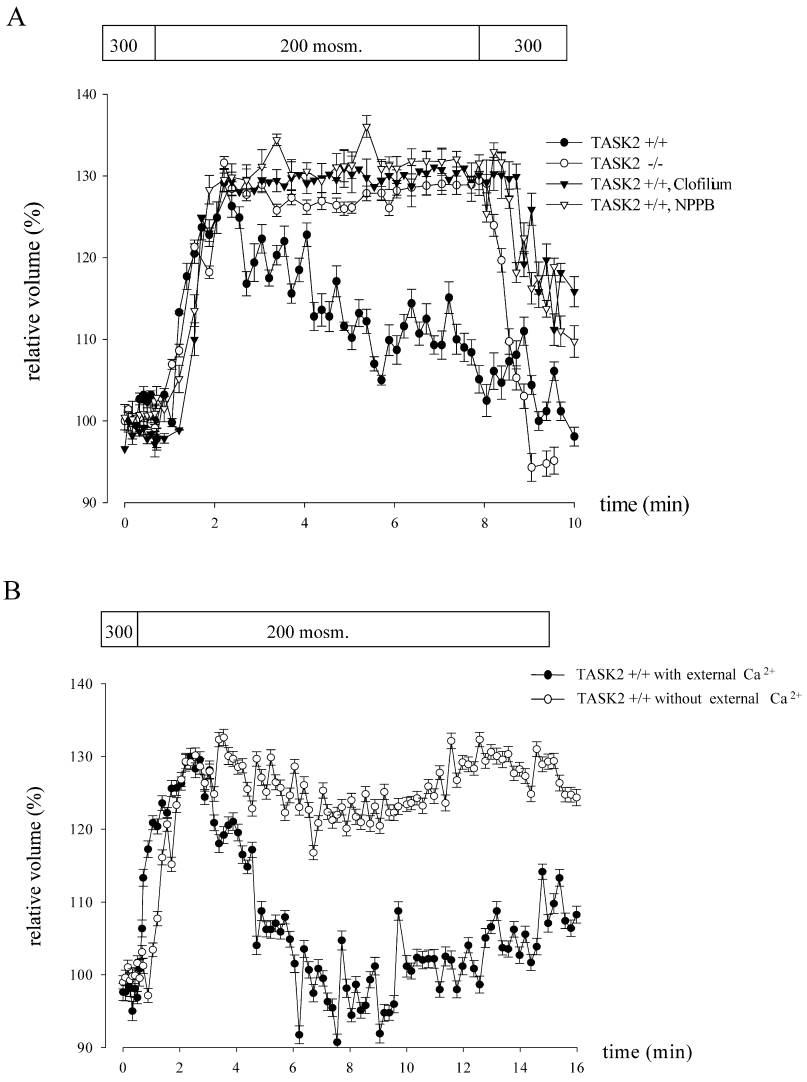

FIGURE 8. RVD in cultured PCT cells from *TASK2* +/+ and *TASK2* −/− mice. After loading with Fura2, cultures were rinsed in 300 mosmol/kg H_2O NaCl solution, and fluorescence of Fura2 was measured at 360 nm for a control period of 1 min. Then, a hypotonic shock was induced by perfusing a 200-mosmol/kg H_2O NaCl solution. Images were recorded every 5 s. After analysis, relative volume change in percentage of initial volume was plotted against time as explained in MATERIALS AND METHODS. (A) Effect of a hypotonic shock on primary cultured cells. Measurements were performed on six monolayers (25 random cells each) from *TASK2* −/− and five monolayers (25 random cell each) from *TASK2* +/+. (B) Effect of external Ca^{2+} on RVD in PCT cells from TASK2 +/+ mice. Measurements were performed on four monolayers (25 random cells each) from *TASK2* +/+ mice.

mV, n = 13 cells from five mice). Whatever the cell type, the swelling-activated K^+ currents were strongly blocked when the cells were reexposed to hypertonic solution (350 mosmol/kg H_2O) as shown in Fig. 10 A (c) and Fig. 10 B (c).

As concerns *TASK2* −/− mice, the effect of hypotonic shock always induced swelling-activated K^+ currents in DCT cells (n = 4; Fig. 10 B). Overall, the results indicate that TASK2 protein could be implicated in the control of swelling-activated K^+ conductances only in proximal segments of the nephron.

RVD in DCT Cells from TASK2 +/+ and TASK2 −/− Mice

To confirm the fact that TASK2 was not implicated in cell volume regulation, we measured relative cell volume in monolayers by fluorescence video microscopy in DCT cells from *TASK2* +/+ and *TASK2* −/− mice. As expected, DCT cells swelled in response to a hypotonic shock (Fig. 10 C). This cell swelling was followed by an RVD in both cell types. 2 min after the hypotonic shock, relative cell volume reached 120 ± 1% (n = 8 monolayers) and 123 ± 2% (n = 4 monolayers) of the initial volume in DCT cells from *TASK2* +/+ and *TASK2* −/− mice, respectively. These cells recovered their initial volume within 2–3 min.

DISCUSSION

Although renal tissue expressed abundant mRNAs encoding the two-pore domain channel (TASK2), nothing is known concerning the physiological role of this channel in the kidney. It is indeed well known that TASK2 generates K^+ currents that are activated by physiological increases in external pH, suggesting that these channels could be particularly efficient in epithelium implicated in pH regulation. Therefore, in the present work, we developed primary cultures of nephron segment microdissected from mice carrying null mutation of the TASK2 gene.

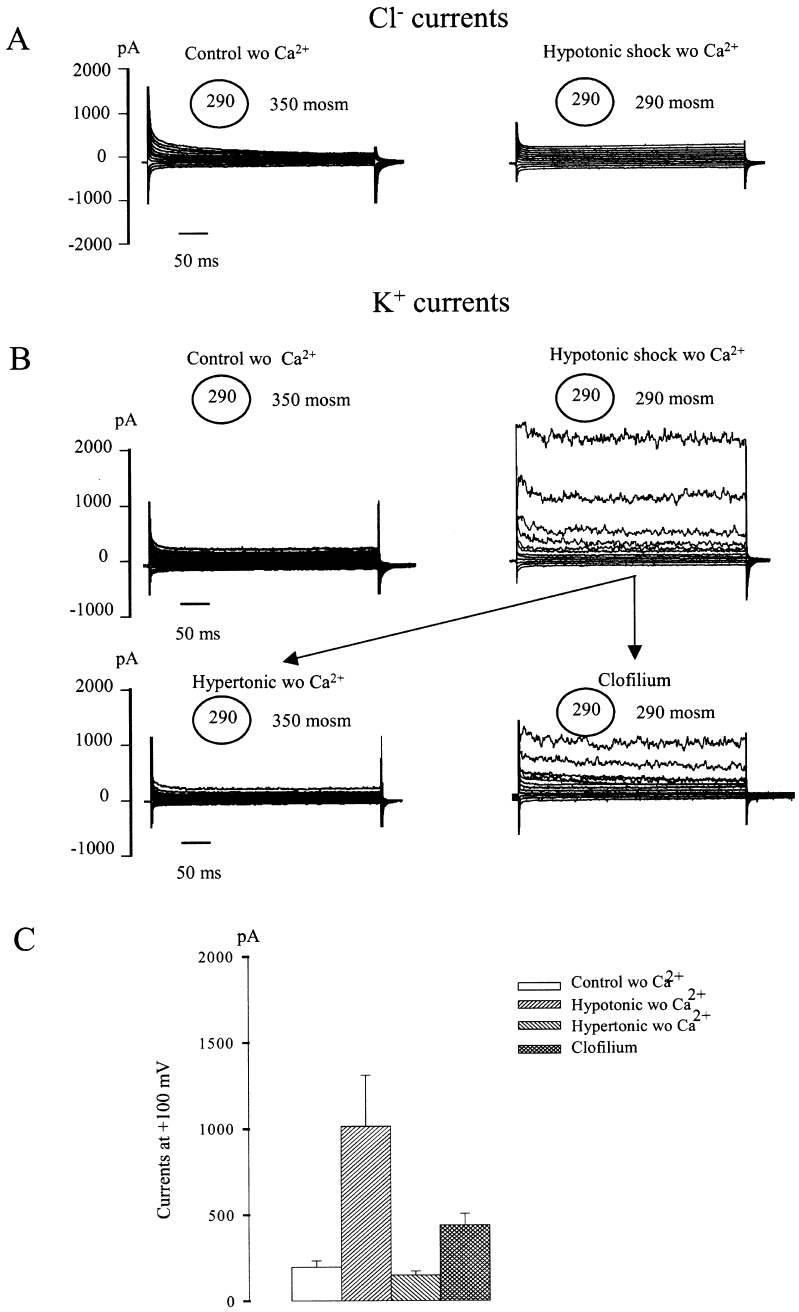

FIGURE 9. Effect of external Ca^{2+} on swelling-activated Cl^- and K^+ currents in primary cultures of PCT cells from *TASK2* +/+ mice. Under each experimental condition, the circled values represent the osmotic pressure in the pipette, and the values outside the circle represent the osmotic pressure in the extracellular bath solutions. (A) Characteristics of swelling-activated Cl^- currents activated by a hypotonic shock in the absence of external Ca^{2+}. Whole-cell currents were recorded in solutions containing 140 mM NMDG-Cl both in the pipette and in the extracellular bath. Membrane voltage was held at −50 mV and stepped to test potential values between −100 and +120 mV in 20-mV increments. (B) Characteristics of swelling-activated K^+ currents activated by a hypotonic shock in the absence of external Ca^{2+}. Whole-cell currents were recorded in solutions 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 and +120 mV in 20-mV increments. (C) Histogram of current values at 100 mV of swelling-activated K^+ currents in different conditions. Values were measured 200 ms after the onset of pulse. Each value is mean ± SEM of 10 cells obtained from five monolayers.

Replacing the TASK 2 gene by the gene encoding β-galactosidase allowed an indirect localization of TASK2 protein. Surprisingly, we found that TASK2 was expressed in cortical PCT in situ and in cultured proximal cells in vitro. This localization is at variance with that reported by Reyes et al. (1998). They identified TASK2 mRNA in the cortical distal tubules and collecting ducts of human adult kidney using in situ hybridization (Reyes et al., 1998). Apart from a difference in the techniques used to detect TASK2, a species difference could explain these conflicting results, but we have no formal arguments to sustain this hypothesis. Nevertheless, the results that we obtained using whole-cell clamp technique clearly indicated that PCT cells from wild-type mice exhibited a K^+ conductance that closely resembled TASK2. Thus, this conductance was insensitive to Ca^{2+}, unaffected by known K^+ channel inhibitors such as TEA or CTX, but was markedly blocked by clofilium and quinidine. Furthermore, these currents presented a strong dependence on the external pH, being activated at alkaline pH in the physiological range variations. The loss of pH-sensitive K^+ currents observed in PCT cells from TASK2 −/− mice definitively indicated that TASK2 is functional in the PCT from wild mice. The TASK2-null mutation not only abolished the pH dependence of the K^+ conductance, but also decreased the control K^+ cur-

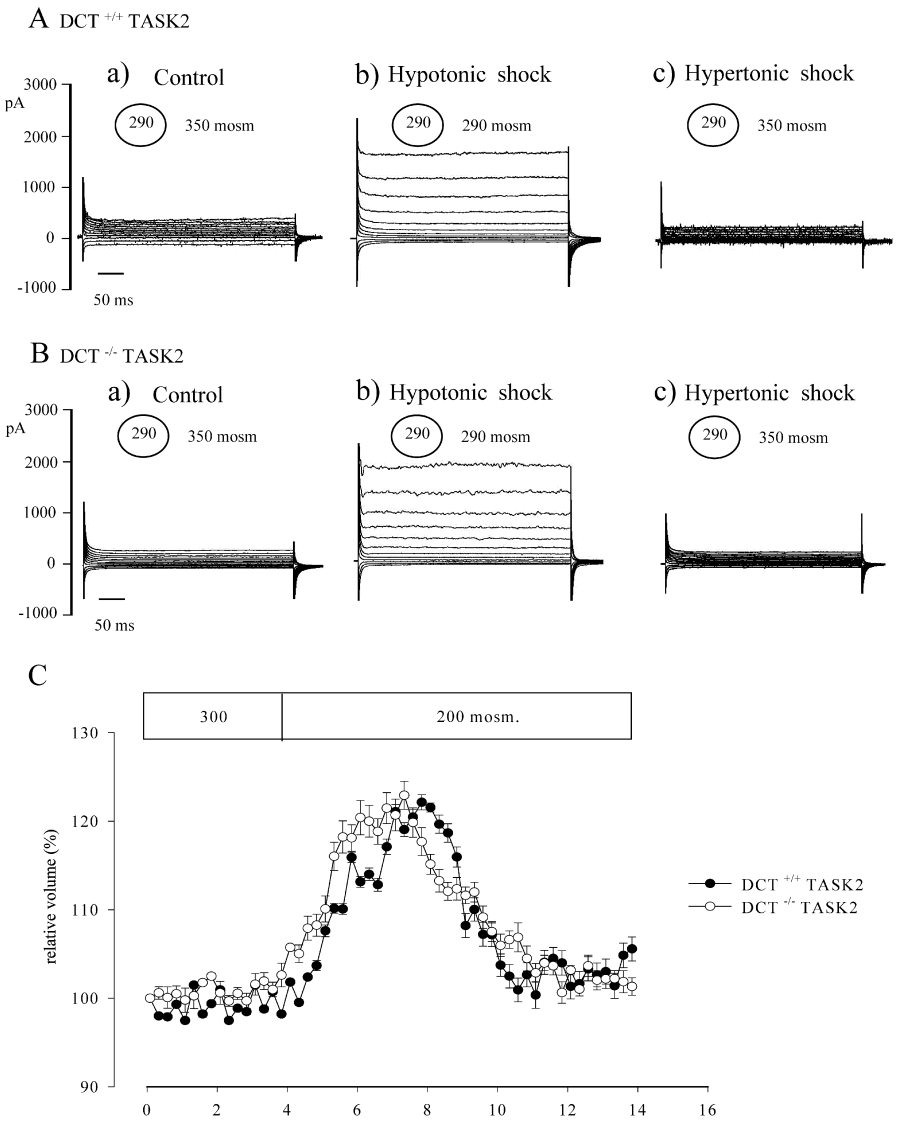

FIGURE 10. Effect of hypotonicity in cultured DCT cells from *TASK2* +/+ and *TASK2* −/− mice. (A and B) Characteristics of swelling-activated whole-cell K^+ currents in primary cultures of DCT cells from *TASK2* +/+ and *TASK2* −/− mice. Under each experimental condition, the circled values represent the osmotic pressure in the pipette, and the values outside the circle represent the osmotic pressure in the extracellular bath solutions. Whole-cell currents K^+ were recorded in solutions 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 and 120 mV in 20-mV increments. (a) Whole-cell currents recorded in control conditions (350 mosmol/kg H_2O in the extracellular bath) after the mechanical rupture of membrane. (b) Whole-cell currents recorded 4–5 min after extracellular perfusion of a 290-mosmol/kg H_2O hypotonic solution. (c) Whole-cell currents were recorded 4 min after extracellular perfusion of a 350 mosmol/kg H_2O hypertonic solution. Values are means ± SEM of four different monolayers from four different mice. (C) RVD in cultured DCT cells from *TASK2* +/+ and *TASK2* −/− mice. After loading with Fura2, cultures were rinsed in 300 mosmol/kg H_2O NaCl solution, and fluorescence of Fura2 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 percentage of initial volume was plotted against time as explained in MATERIALS AND METHODS. Measurements were performed on six monolayers (25 random cells each) from *TASK2* −/− and five monolayers (25 random cell each) from *TASK2* +/+.

rents measured at a pH of 7.4. Therefore, it is probable that TASK2 currents could participate in the control of the negative resting membrane potential. Consequently, absence of this current in the proximal cells of TASK2 −/− mice could result in a slight cell depolarization. The effect of this depolarization on overall renal function is not yet known. However, recent experiments by Warth et al. (2002) using clearance measurements in TASK2 KO mice show an increase in urinary flow rate and salt loss. This could decrease the ability of the proximal cell membrane to repolarize during $NaHCO_3$ reabsorption (Warth et al., 2002), leading to a reduction of electrogenic Na^+/HCO_3^- cotransport.

To further analyze the role of TASK2 channels in the tissue, we addressed the possibility that these channels be implicated in the swelling-sensitive K^+ conductance that was previously found in PCT cells (Belfodil et al., 2003). In these series, the control experiments (pharmacological pattern, pH sensitivity, and the effect of external Ca^{2+}) were performed in TASK2 +/+ mice. These animals exhibit the same genetic background as the one of control mice used for CFTR study by Belfodil et al. (2003). Therefore, it is not surprising that the results obtained in these experimental series are confirmatory to those previously shown by Belfodil et al. (2003). In fact, several studies in Ehrlich cells provided

some evidence that among the K^+ channels involved in the RVD process, TASK2 could be a putative candidate (Hoffmann, 2000). Moreover, a recent report by Niemeyer et al. (2001a) clearly supports this hypothesis by demonstrating that the transfection of TASK2 into HEK 293 cells induced swelling-activated K^+ currents. Interestingly, the K^+ conductance activated by hypotonic challenge in PCT cells from wild-type mice shared many characteristics with the K^+ currents flowing through TASK2 channels. These swelling currents were resistant to conventional K^+ blockers, although they were inhibited by clofilium and quinidine. They were also Ca^{2+} independent. Moreover, increasing the extracellular pH resulted in a strong increase of conductance for the duration of the hypotonic shock. The observation that the null mutation of TASK2 completely suppressed the development of the swelling-activated K^+ conductance further demonstrates that TASK2 is responsible for the overall K^+ currents activated by hypotonic shock in PCT cells in primary culture. In fact, in PCT (as in many tissues), RVD is mainly mediated by a parallel activation of K^+ and Cl^- channels (Tauc et al., 1990). Thus, the lack of swelling-activated K^+ conductance in PCT cells from TASK2 $-/-$ mice could explain why these cells did not exhibit RVD process, because swelling-sensitive Cl^- channels were unaffected by the null mutation. Although TASK2 currents are quite insensitive to Ca^{2+}, it should be noted that in the absence of external Ca^{2+}, the RVD was completely impaired in PCT cells from TASK2 $+/+$ mice. This loss of volume regulation was mainly due to an inhibition of the swelling-activated Cl^- channels, the activation of which depends on external Ca^{2+} (Tauc et al., 1990; Barriere et al., 2003).

Different types of K^+ conductances are activated during cell swelling (le Maout et al., 1990; Hoffmann and Hougaard, 2001; Niemeyer et al., 2001b), and it is interesting to know whether various swelling-sensitive K^+ channels could be activated in the same cell. Recently, Hoffmann proposed a very stimulating model to explain the RVD response in Ehrlich cells (Hoffmann, 2000). According to her hypothesis, hypotonic swelling could result in the activation of two types of K^+ channels: the cells could undergo a standard RVD due to the activation of TASK2 K^+ channels, followed by a potentiated RVD due to the activation of Ca^{2+}-sensitive K^+ channels. However, in renal epithelial cells, the K^+ channels involved in RVD are probably different from one segment to another. In PCT, our data indicate that RVD corresponded to the "standard type" and was mediated by TASK2 K^+ channels, whereas in DCT and CCT cells that do not express TASK2 protein, RVD was mediated by Ca^{2+}-sensitive K^+ channels belonging to Ca^{2+}-activated maxi K^+ channels and Ca^{2+}-activated small K^+ channels (Belfodil et al., 2003).

In conclusion, the present report clearly demonstrates that TASK2 K^+ channels are functional in PCT cells. At rest, these channels could participate in the control of the membrane potential. Importantly, TASK2 is the main K^+ conductance involved in the RVD process in PCT.

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