Regulation of the Hyperpolarization-activated K^+ Channel in the Lateral Membrane of the Cortical Collecting Duct

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ABSTRACT An intermediate-conductance K^+ channel (I.K.), the activity of which is increased by hyperpolarization, was previously identified in the lateral membrane of the cortical collecting duct (CCD) of the rat kidney (Wang, W. H., C. M. McNicholas, A. S. Segal, and G. Giebisch. 1994. *American Journal of Physiology*. 266:F813–F822). The biophysical properties and regulatory mechanisms of this K^+ channel have been further investigated with patch clamp techniques in the present study. The slope conductance of the channel in inside-out patches was 50 pS with 140 mM KCl in the pipette and 5 mM KCl, 140 mM NaCl (NaCl Ringer's solution) in the bath. Replacement of the bath solution with symmetrical 140 mM KCl solution changed the slope conductance of the channel to 85 pS and shifted the reversal potential by 55 mV, indicating that the selectivity ratio of K^+/Na^+ was at least 10:1. Channel open probability (P_o) in inside-out patches was 0.12 at 0 mV and was increased by hyperpolarization. The voltage-dependent P_o was fitted with the Boltzmann's equation: $P_o = 1/[1 + \exp(V - V_{1/2})zF/RT]$, with $z = 1.2$ and $V_{1/2} = -40$ mV. Addition of 2 mM tetraethylammonium or 500 mM quinidine to the bath blocked the activity of the K^+ channel in inside-out patches. In addition, decrease in the bath pH from 7.40 to 6.70 reduced P_o by 30%. Addition of the catalytic subunit of protein kinase A (PKA_c; 20 U/ml) and 100 mM MgATP to the bath increased P_o from 0.12 to 0.49 at 0 mV and shifted the voltage dependence curve of channel activity toward more positive potentials by 40 mV. Two exponentials were required to fit both the open-time and the closed-time histograms. Addition of PKA_c increased the long open-time constant and shortened the long closed-time constant. In conclusion, PKA-mediated phosphorylation plays an important role in the regulation of the voltage dependence of the hyperpolarization-activated K^+ channel in the basolateral membrane of CCD.

INTRODUCTION

The cortical collecting duct (CCD) is a nephron segment important for K^+ secretion and hormone-regulated Na^+ reabsorption (Stanton and Giebisch, 1992; Breyer and Ando, 1994). Two types of cells, principal and intercalated cells, have been identified in the CCD (O'Neil and Hayhurst, 1985). It is well established that

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the principal cell is responsible for the K^+ secretion and Na^+ reabsorption. Early studies with microelectrodes have established the cellular mechanisms of the K^+ secretion and the Na^+ reabsorption (Koeppen, Biagi, and Giebisch, 1983). Na^+ ions enter the cell through luminal Na^+ channels, and Na^+ ions are then pumped out of the cell via an Na^+/K^+-ATPase. For K^+ secretion, K^+ ions are taken up by the Na^+/K^+-ATPase and are subsequently secreted into the lumen via ATP-sensitive low-conductance K^+ channels (Wang, Schwab, and Giebisch, 1990; Frindt and Palmer, 1989; Ling, Hinton, and Eaton, 1991; Schlatter, 1993). A significant K^+ conductance is present in the basolateral membrane of the CCD. At least three important functions are served by the K^+ channels in the basolateral membrane. First, they participate in generating the cell membrane potential. Because both the Na^+ reabsorption and K^+ secretion are electrogenic processes, alteration of the membrane potential can have a profound effect on the rates of Na^+ reabsorption and K^+ secretion. Second, they are responsible for K^+ recycling across the basolateral membrane. Finally, the basolateral K^+ channels can provide an additional route for K^+ entering the cell, when the membrane potential is sufficiently hyperpolarized to exceed the equilibrium potential for K^+ (Sansom and O'Neil, 1986). Microscopic electrophysiological investigations have revealed that the basolateral K^+ conductance is closely coupled to the activity of the Na^+/K^+-ATPase (Horisberger and Giebisch, 1988). When Na^+ reabsorption is enhanced, an increase of the basolateral K^+ conductance is observed after stimulation of the Na^+/K^+-ATPase (Beck, Hurst, Lapointe, and Laprade, 1993; Hurst, Beck, Laprade, and Lapointe, 1991). Thus, these results provide additional evidence of the importance of basolateral K^+ channels in Na^+ reabsorption and K^+ secretion.

Although the basolateral K^+ channels play an important role in maintaining the normal function of the CCD principal cells, little information is available concerning their biophysical properties and regulatory mechanisms. Recently, efforts have been made to study the K^+ channels in the basolateral membrane of the CCD by two different approaches (Wang, McNicholas, Segal, and Giebisch, 1994; Hirsch, and Schlatter, 1993). It is clear from those two studies that at least two types of K^+ channels, a low-conductance channel (28 pS) and an intermediate-conductance (I.K.) channel (50–90 pS), are present in the basolateral membrane of the CCD. In previous studies, it was demonstrated that the low-conductance K^+ channel has a high open probability (0.8) and is not voltage dependent. In contrast, the open probability for the I.K. channel is voltage dependent such that hyperpolarization activates channel activity. In this study the biophysical properties and the regulation of the I.K. channel are further explored.

METHODS

Preparation of Cortical Collecting Duct Tubules

Sprague-Dawley rats of either sex (40–50 g) were obtained from Taconic Farm Inc. (Germantown, NY) and maintained in a pathogen-free room for at least 1 wk before experiments. After the animals were killed, the kidneys were immediately removed and the thin coronal sections were cut with a razor blade. Kidney slices were immersed in HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid)-buffered NaCl Ringer's solution containing (in millimolar) 140 NaCl, 5 KCl,

1.8 $MgCl_2$, 1.8 $CaCl_2$, 5 glucose, 10 HEPES (pH 7.4). CCD tubules were manually dissected and transferred onto a 5 × 5 mm cover glass coated with Cell-Tak (Collaborative Research Inc., Bedford, MA) to immobilize the tubules. The cover glass was then placed in a chamber (1,000 ml/vol) mounted on an inverted microscope (Nikon Diphot) with Hoffman optics. The tubules were subsequently cut open longitudinally with a sharpened glass pipette, and the edges of the opened tubule were pressed against the cover glass to form a monolayer with the apical cell membrane facing upward. The method for exposure of the lateral surface of the principal cell has been described previously (Wang et al., 1994). Briefly, after the CCD was split, the tubule was incubated in Ca^{2+}-free Ringer's solution for 10 min. A pipette (2 mm) was mounted on the manipulator (Narishige) and moved to the surface of the intercalated cell adjoining an identified principal cell. Negative pressure was applied to the pipette using mouth suction such that a firm attachment between the pipette and the intercalated cell could be formed. Then the pipette was slowly raised until a complete dissociation of the intercalated cell from the CCD tubule was achieved. The bath solution was switched to the Ca^{2+}-containing Ringer's solution, and the temperature of the chamber was maintained at 37 ± 1°C by circulating warm water in a jacket surrounding the chamber.

Patch Clamp Recordings

The patch clamp amplifier (Axon 200A; Axon Instruments, Inc., Foster City, CA) was used in the voltage clamp mode. Patch electrodes were pulled from a vertical pipette puller (model 700C, David Kopf Instruments, Tujunga, CA) in two stages using glass capillaries (Dagan, Minneapolis, MN) and fire polished to resistances of 4–6 MΩ when filled with 140 mM KCl. The output of the patch clamp amplifier was low pass filtered at 1 kHz through an eight-pole Bessel filter (Frequency Devices, Inc., Haverhill, MA), digitized at a sampling rate of 44 kHz using a modified Sony PCM-501ES pulse code modulator, and stored on videotape (Sony SL-2700). For analysis, data were acquired and written to a hard disk (Gateway 2000 4DX) at a sampling rate of 5 kHz and analyzed using pCLAMP software (version 6.01; Axon Instruments, Inc.). Opening and closing transitions were detected using 50% of the single-channel amplitude as the threshold. Channel activity was expressed as the sum of the fractional open time (NP_o) at each channel current level (1–N) and calculated by the equation

$$NP_o = \sum_{n=1}^{N} T_n \quad (1)$$

where N is the number of observed current levels in the patch, and T_n is the fractional open time spent at each of the current levels. NP_o was determined from data acquired during a 30–60-s period. The method for obtaining the mean open probability (P_o) and the slope conductance of the channel has been described previously (Cassola, Giebisch, and Wang, 1993). The maximum likelihood method with simplex optimization was used to fit the open- and closed-time histograms with sums of exponential probability density functions. The fit was corrected for the dead time, T_d, of the recording system, which was estimated from the formula $T_d = 0.179/f_c$, where f_c is the filter cut off frequency in hertz (Colquhoun and Sigworth, 1983). The dead time was thus estimated to be 0.2 ms at 1,000 Hz.

Experimental Solutions

The pipette solution contained (in millimolar): 140 KCl, 1.8 $MgCl_2$, 1 EGTA (ethyleneglycol-bis-(b-aminoethylether) *N,N,N'N'*-tetraacetic acid), and 10 HEPES (pH 7.40). The Ca^{2+}-free NaCl Ringer's solution for inside-out patches contained (in millimolar): 140 NaCl, 5 KCl, 1.8 $MgCl_2$, 1 EGTA, and 10 HEPES (pH 7.40 or 6.70); the Mg^{2+}- and Ca^{2+}-free NaCl Ringer's solution contained (in millimolar): 140 NaCl, 5 KCl, 1 EGTA, 1 EDTA (ethylenediaminetetraacetic acid), and 10 HEPES (pH 7.4 or 6.7).

Statistics

Data are shown as mean ± SD of the mean (*n*), where *n* is the observation number. A paired *t* test was used to determine the significance of differences between the experimental groups. Statistical significance was taken as $P < 0.05$.

RESULTS

We have confirmed previous observations that the I.K. channel is activated by hyperpolarization (Wang et al., 1994). Fig. 1 *A* is a typical single-channel recording made from an inside-out patch showing voltage-dependent channel open probability (P_o). It is apparent that P_o of the channel is lower at positive holding potentials (depolarization) than at negative holding potentials (hyperpolarization). P_o increased from 0.04 ± 0.01 to 0.85 ± 0.04 (n = 4) when the holding potential was changed from 30 to −65 mV. The hyperpolarization-induced stimulation of the channel activity could also be observed in a Mg^{2+}-free solution, indicating that the voltage dependence of activation of I.K. is not a result of Mg^{2+}-induced blockade (data not shown). The figure shows two types of channel opening events, a fast, burstlike opening and a long-lasting opening. Hyperpolarization increased the frequent occurrence of the long-lasting openings. Fig. 1 *B* shows the kinetic analysis of the I.K. At least two time constants are required to fit both the open- and closed-duration histograms. Hyperpolarization not only reduced the probability for the channel being in the long closed state, but also shortened the long closed time constant (Table I). In contrast, both the probability for the channel staying in the long open state and the long open-time constant were significantly increased by hyperpolarization (Fig. 1 *B* and Table I).

This hyperpolarization-activated K^+ channel was not inactivated for several seconds upon stimulation by a hyperpolarization pulse. Fig. 2 is a representative channel recording made from an inside-out patch with Ca^{2+}-free NaCl Ringer's solution in the bath. It shows the response of channel activity to a series of hyperpolarization pulses (−60 mV). It is apparent that the channel activity was maintained during the whole time period of the hyperpolarization pulse (1,800 ms). However, the activity of the K^+ channel decreased slowly when the membrane potential was clamped at the same voltage for several minutes. Fig. 3 is a typical example demonstrating the slow inactivation after activation of the K^+ channel by −45 mV hyperpolarization. Within an average of 150 ± 20 s (N = 4), P_o decreased to the midpoint between the peak and steady state values (data not shown). This decrease in channel activity was not the result of channel rundown, because channel activity was restored when the cell membrane potential was depolarized to the original holding potential followed by rehyperpolarization to the same level as before. Thus, the data suggest that the hyperpolarization-gated K^+ channel in the CCD does not have fast channel inactivation. The hyperpolarization-activated K^+ channel has a high selectivity for K^+. Fig. 4 shows *I–V* curves made in inside-out patches. The curve has a slope conductance of 50 ± 2 pS (N = 26) in the range between −20 and 0 mV with 5 mM KCl, 140 mM NaCl in the bath. When the bath K^+ concentration was increased from 5 to 140 mM, the extrapolated reversal potential was shifted by ~55 mV and the calculated selectivity ratio between K^+ and Na^+ was

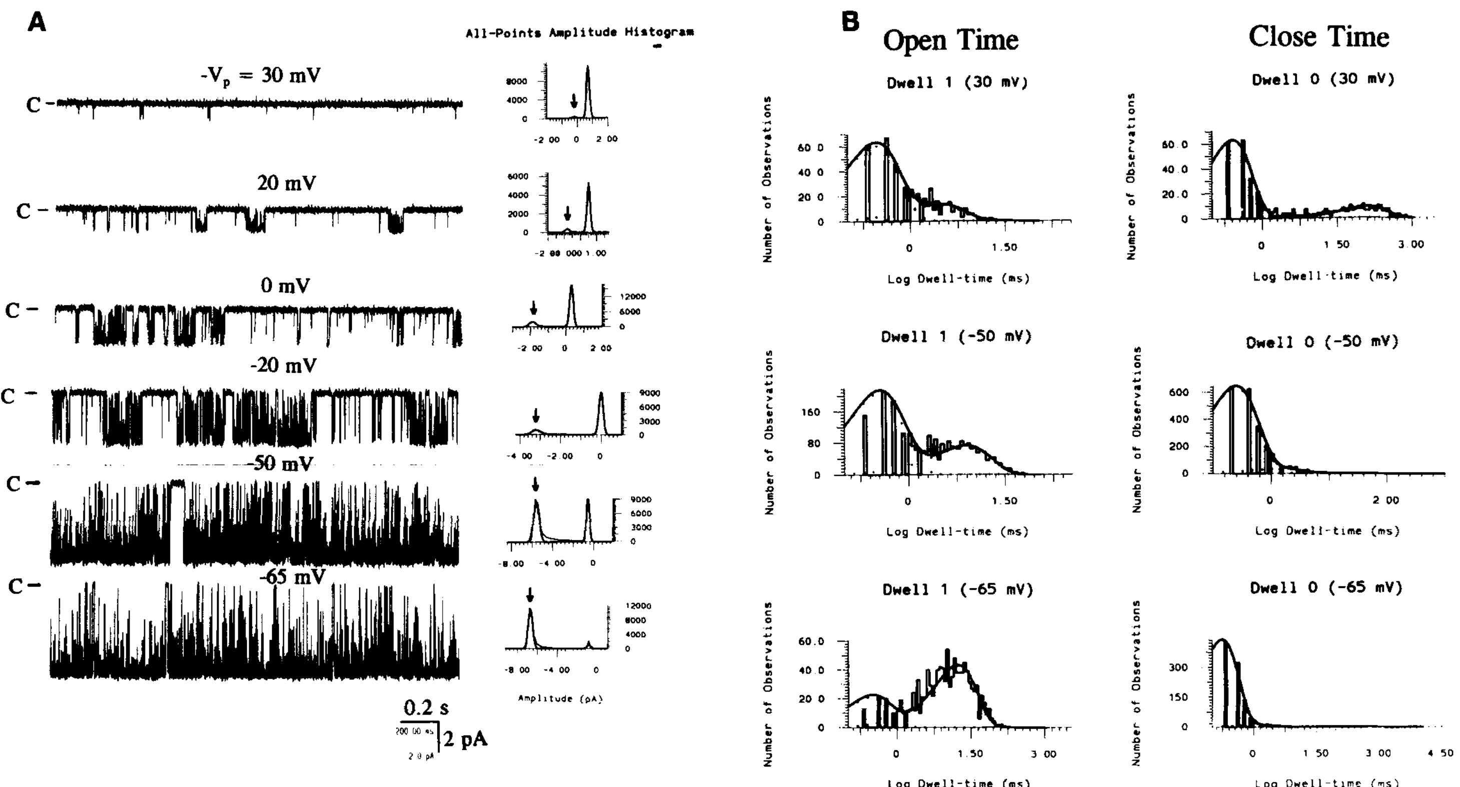

FIGURE 1. (*A*) A channel recording made in an inside-out patch with Ca^{2+}-free NaCl Ringer's solution in the bath. The membrane potentials are indicated at the top of the tracing, and the channel closed level is indicated by *C*. The right side of the figure shows the all-points amplitude histograms, and the channel current in the open state is indicated by an arrow. (*B*) The open- and closed-time histograms of I.K. at different membrane potentials. Note that hyperpolarization increases the area of the long open time and decreases that of the long closed time.

TABLE I

Open- and Closed-Time Constants and Channel Open Probability

	τ_o1	τ_o2	τ_c1	τ_c2	P_o	N
mV			*ms*			
30	0.3 ± 0.05	2.6 ± 0.12	0.3 ± 0.04	122 ± 1.0	0.04 ± 0.01	4
0	0.4 ± 0.08	4.4 ± 0.1*	0.4 ± 0.08	27 ± 0.2*	0.12 ± 0.02*	4
–50	0.4 ± 0.05	7.0 ± 0.06*	0.4 ± 0.03	3 ± 0.1*	0.62 ± 0.03*	4
–65	0.5 ± 0.05	14 ± 0.1*	0.4 ± 0.04	2.2 ± 0.1*	0.85 ± 0.04*	4

Data were obtained from inside-out patches. τ_o is the open-time constant; τ_c is the closed-time constant. Asterisks indicate values significantly different ($P < 0.01$) from the value at 30 mV.

>10. In addition, the *I–V* curve became linear and yielded a conductance of 85 ± 3 pS ($N = 4$).

This K^+ channel was sensitive to tetraethylammonium (TEA) as well as Ba^{2+}. Fig. 5 *A* is a recording made from an inside-out patch showing the inhibitory effect of 2 mM TEA applied to the bath. Inspection of Fig. 5 *A* demonstrates that TEA does not reduce channel P_o, but the apparent channel currents were significantly decreased. Characteristics of TEA-induced inhibition of channel activity indicate that TEA is a fast channel blocker for the hyperpolarization-activated K^+ channel (Hille, 1992). The TEA-induced inhibition is voltage dependent. Fig. 5 *B* shows the apparent channel current as a function of voltage under control conditions and in the presence of TEA. The voltage dependence of internal TEA block was fitted by the Hille (1992) equation:

$$I_i = I_{Control}\,[1 + [B][K_0\exp(F\delta V/RT)]^{-1} \quad (2)$$

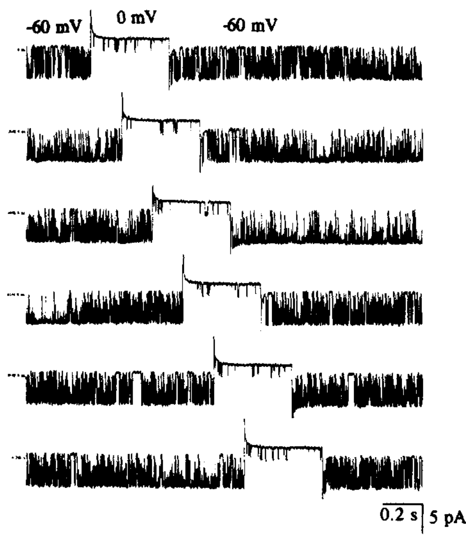

FIGURE 2. A channel recording made in an inside-out patch with Ca^{2+}-free NaCl Ringer's solution in the bath. The membrane potentials are clamped at 0 mV for 500 ms and then hyperpolarized to −60 mV for 1,800 ms. Note that no fast inactivation of channel activity upon hyperpolarization occurs.

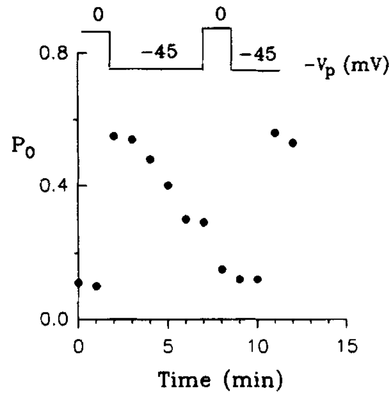

FIGURE 3. The time-dependent channel open probability at 0 and −45 mV. P_o increases promptly in response to hyperpolarized voltage and decreases gradually at the same membrane potential.

where I_i and $I_{Control}$ are the currents measured in the presence of inhibitor (TEA) and in the absence of TEA, respectively, B is the inhibitor concentration, K_0 is the 0-voltage equilibrium constant for the inhibitor, V is the pipette holding potential, and δ is the fraction of the voltage sensed at the TEA-binding site. It is apparent that depolarization enhances the TEA-induced inhibition and the calculated voltage dependence of internal TEA is high ($\delta = 0.83 \pm 0.03$, $N = 3$). The calculated value of K_0 for internal TEA is 1.15 ± 0.1 mM ($N = 3$; Fig. 5 *C*).

The activity of the hyperpolarization-activated K^+ channel was also sensitive to quinidine. Fig. 6 is a channel recording made in an inside-out patch demonstrating inhibition of channel activity by quinidine applied to the bath. As with TEA, the

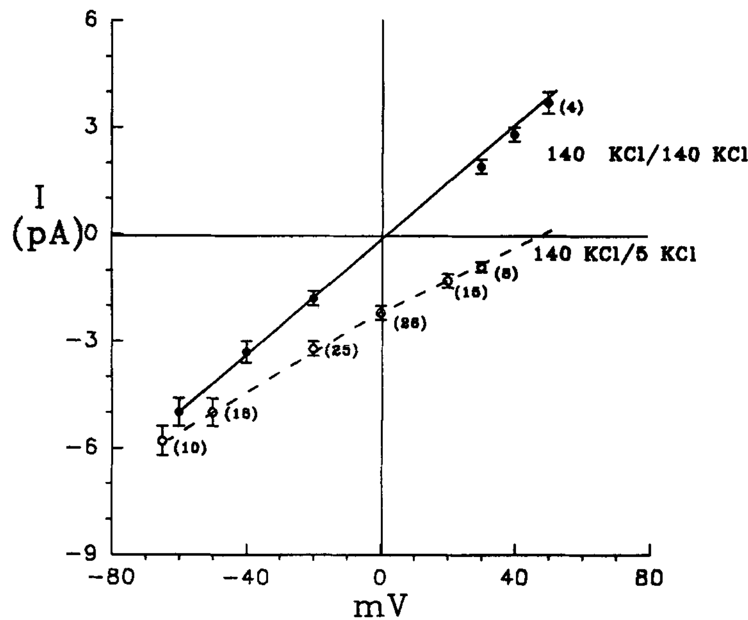

FIGURE 4. *I–V* curve of I.K. with symmetrical 140 mM KCl solution (*filled circles*) or with 140 mM KCl in the pipette and 5 mM KCl (Ca^{2+}-free NaCl Ringer's solution) in the bath (*open circles*).

block induced by 0.5 mM quinidine appears as a decrease in the single-channel current amplitude (Fig. 6, *A* and *B*). Thus, block and unblock events must be too rapid to be detected, as suggested by Hille (1992). The quinidine-induced inhibition is slightly voltage dependent. Fig. 6 *C* shows a curve of the apparent channel current vs voltage under control conditions and in the presence of quinidine (0.5 mM). The apparent channel current was measured after the data were refiltered at 50 Hz (Fig. 6 *B*). The voltage dependence of internal quinidine block was fitted by Eq. 2 (Fig. 6 *D*), and the calculated K_o for internal quinidine was 0.48 ± 0.04 mM

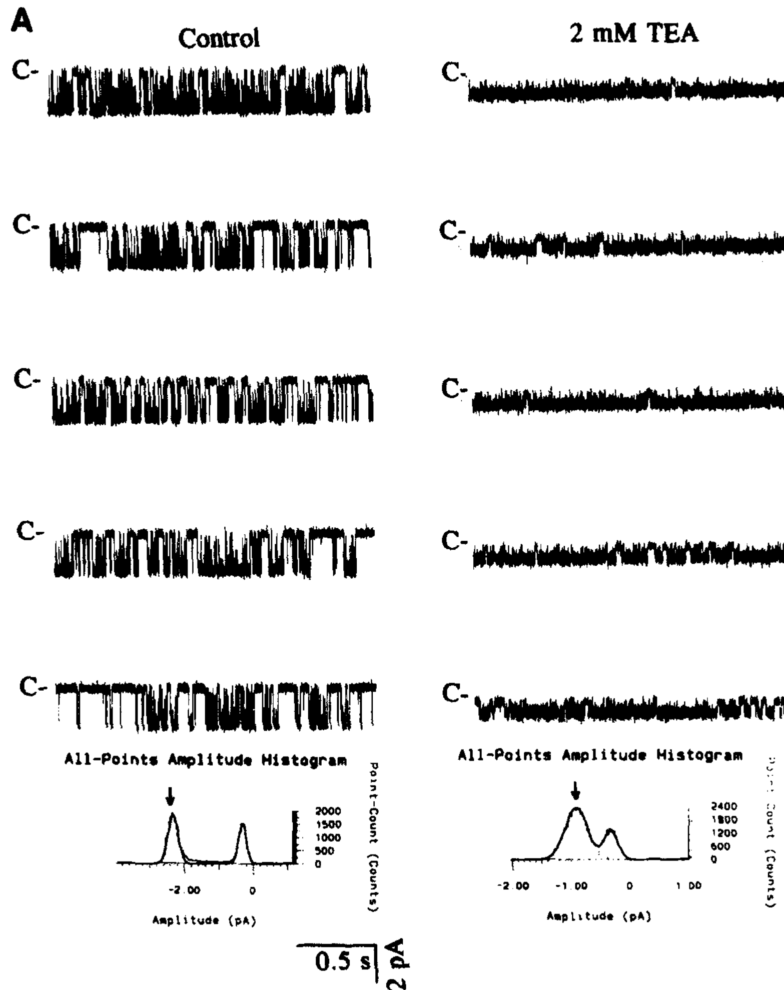

FIGURE 5. (*A*) A typical channel recording was made in an inside-out patch with symmetrical 140 mM KCl solution, showing the effect of 2 mM TEA added to the bath on the activity of I.K. The membrane potential was −25 mV ($-V_p$), and 100 mM MgATP and 10 U/ml PKA catalytic subunit were present in the bath. The all-points amplitude histograms for the activity of I.K under control condition and in the presence of TEA are displayed at the bottom of the figure. (*Arrow*) Channel current in the open state.

($N = 3$). The value of δ for block by internal quinidine was very small (0.06 ± 0.01, $N = 3$).

The hyperpolarization-gated channel was pH sensitive. Fig. 7 shows that a decrease in bath pH from the control value of 7.4 to 6.7 significantly reduced channel activity by $30 \pm 5\%$ ($n = 4$). The effect of pH was fully reversible and was not voltage dependent (data not shown).

cAMP-dependent protein kinase A (PKA) has been shown to play an important role in the regulation of Na^+ reabsorption and K^+ secretion in the CCD (Breyer and Ando, 1994; Field and Giebisch, 1985; Schafer and Troutman, 1990; Wang and Giebisch, 1991). Thus, the effect of PKA on the activity of the hyperpolarization-

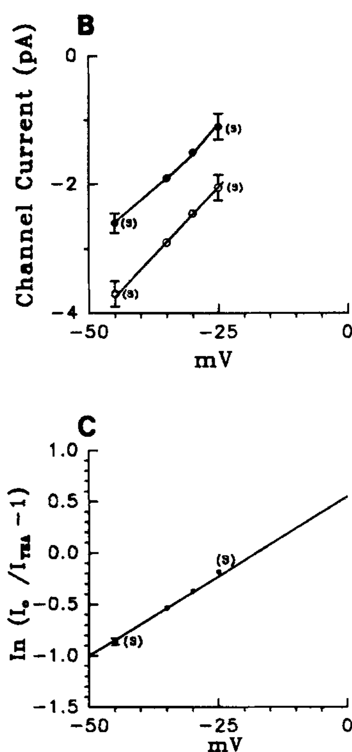

FIGURE 5. (*B* and *C*) Apparent channel currents are plotted against the membrane potentials under control conditions (*open circles*) and in the presence of 2 mM internal TEA (*filled circles*). The voltage dependence of internal TEA block was fitted by Eq. 2. I_o and I_{TEA} are the apparent channel current under control conditions and in the presence of 2 mM TEA, respectively. The calculated K_o for TEA is 1.15 mM, and δ is 0.83 (*C*).

gated K^+ channel was investigated. Fig. 8 *A* is a representative recording made from an inside-out patch and shows that the catalytic subunit of PKA (PKA_c) modulates the voltage-dependent gating of the K^+ channel. Fig. 8 *A* shows that addition of PKA_c significantly increased P_o and switched kinetics of the channel from the burst-like opening to more long-duration events. Fig. 8 *B* further illustrates that addition of PKA_c increased the time constant for long-duration opening from 4.4 ± 0.1 to 8.2 ± 0.1 ms ($N = 4$) and decreased the time constant for long-duration closure from 27 ± 0.2 to 19 ± 0.1 ms ($N = 4$). In contrast, both the time constants for short-duration openings (0.4 ms) and closures (0.3 ms) were not altered by PKA_c (Table II). In addition, P_o for the channel at 0 mV holding potential was significantly increased from 0.12 ± 0.02 before PKA_c to 0.49 ± 0.03 ($N = 4$) after PKA_c

(Table II). The effects of PKA_c on the activity of I.K. were observed in six of six experiments, if PKA_c and MgATP were added before channel rundown. However, PKA_c failed to restore channel activity in patches with run-down channels (data not shown). The effect of PKA_c was dependent on the presence of MgATP, because addition PKA_c alone without MgATP had no stimulatory effect on I.K.

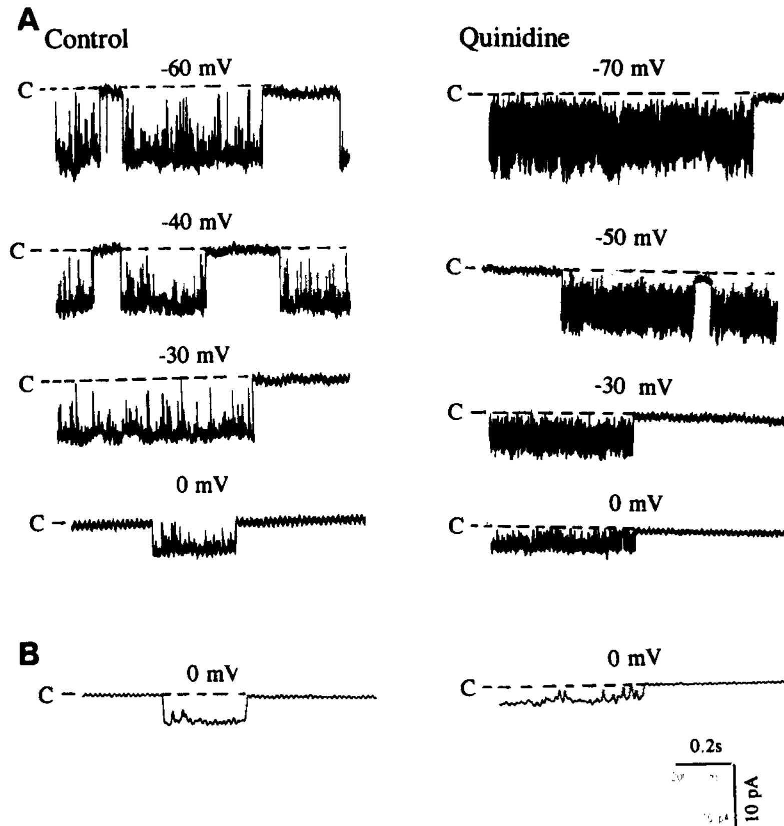

FIGURE 6. Channel current was recorded in an inside-out patch with Ca^{2+}- and Mg^{2+}- free NaCl Ringer's solution in the bath, showing the effect on channel activity of quinidine added to the bath. The closed level is indicated by *C*, and the membrane potential is indicated above each tracing. Data were filtered at either 1,000 Hz (*A*) or 50 Hz (*B*).

Voltage-dependent openings of the channel could be fitted by Boltzmann's equation:

$$P_o = 1/[1 + \exp(V - V_{1/2})zF/RT] \quad (3)$$

where V is the membrane potential (pipette clamping voltage for inside-out patches), $V_{1/2}$ is the membrane potential required to produce a 50% change in the maximum P_o, z is the effective charge, F is the Faraday constant, R is the gas constant, and T is the absolute temperature. The calculated equivalent charge (z) is 1.2

± 0.1, and $V_{1/2} = -40 \pm 2$ mV ($N = 4$) in the absence of PKA (Fig. 9). Addition of PKA did not alter the value of the equivalent charge, but shifted $V_{1/2}$ from -40 ± 2 to 0 ± 2 mV. Thus, PKA decreased the voltage dependence of the channel.

DISCUSSION

Two types of K^+ channels, a low-conductance (28 pS) and an I.K. channel (50 pS), have previously been identified in the lateral membrane of the CCD (Wang et al., 1994). Using the collagenase-treated rat CCD, Hirsch and Schlatter (1993) also

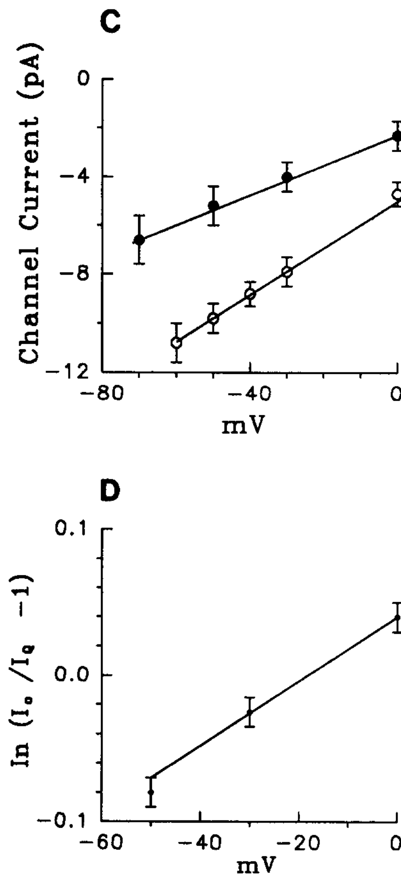

FIGURE 6. (*C* and *D*) Apparent channel currents (obtained by filtering data at 50 Hz) are plotted against the membrane potentials under control conditions (*open circles*) and in the presence of 0.5 mM internal quinidine (*filled circles*). The data points represent the mean of three observations (*C*). The voltage dependence of internal quinidine block was fitted by Eq. 2. The calculated K_d for quinidine is 0.48 mM, and δ is 0.06 (*D*). I_o and I_Q are the apparent channel current under control conditions and in the presence of 0.5 mM quinidine, respectively.

identified two types of K^+ channel in peritubular membrane. The intermediate-conductance K^+ channel observed in their study was shown to be sensitive to pH and TEA. However, no voltage sensitivity of the intermediate-conductance K^+ channel was observed in that study. In addition, the channel conductance in symmetrical KCl solution was larger (200 pS) than that observed in the present study (85 pS). It is not clear whether the discrepancy results from methodologic differences or whether the population of K^+ channels in the lateral membrane is indeed different from that in the peritubular membrane. Further investigation is necessary to re-

solve this discrepancy. However, it is clear that at least two populations of K^+ conductances are present in the basolateral membrane of the CCD. Studies performed on A6 cells, a cell line that shares the functional properties of the CCD, have demonstrated that under control conditions, the basolateral K^+ conductance is Ba^{2+} sensitive but quinidine insensitive (Broillet and Horisberger, 1991). However, cell swelling induced a second type of K^+ current that was inhibited by quinidine (Broillet and Horisberger, 1991). Interestingly, the intermediate-conductance K^+ channel was quinidine sensitive. However, it is not known whether cell swelling increases the activity of the intermediate-conductance K^+ channel.

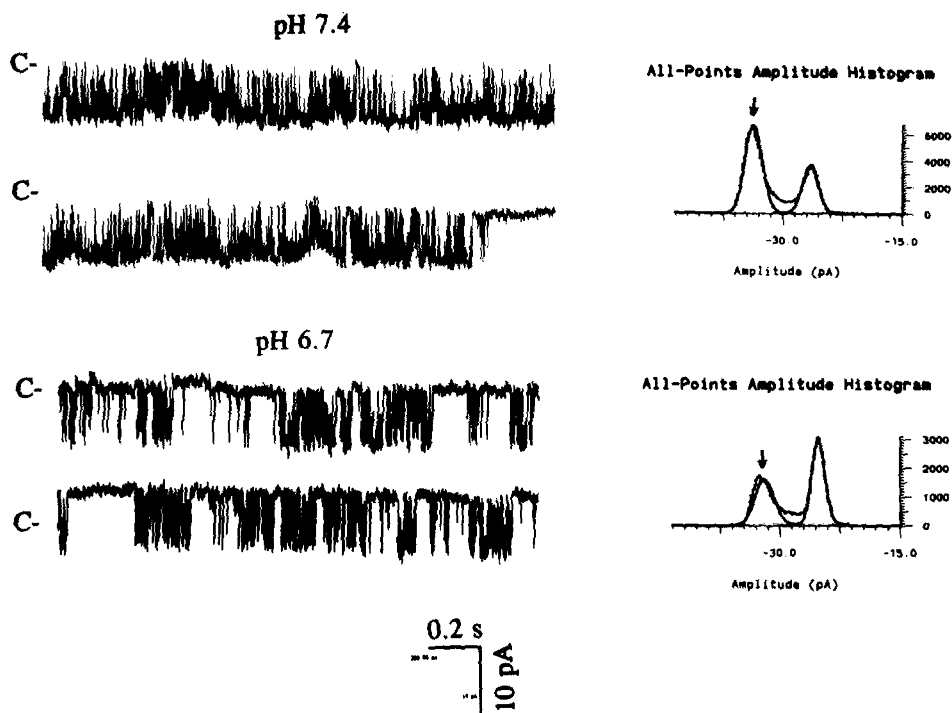

FIGURE 7. Effect of pH on the activity of I.K. in an inside-out patch with Ca^{2+}-free NaCl Ringer's solution in the bath. The channel closed level is indicated by *C*, and the membrane potential is −50 mV. The corresponding all-points amplitude histograms for pH 7.4 and pH 6.7 are shown on the right side of the figure. (*Arrow*) Channel current in the open state.

Several lines of evidence suggest that the hyperpolarization-activated K^+ channel may not be mainly responsible for the "basal" K^+ conductance in the basolateral membrane. First, P_o of the hyperpolarization-activated channel is 0.20 at the spontaneous cell membrane potential, whereas that of the low-conductance K^+ channel is 0.80 (Wang et al., 1994). Second, NP_o of the low-conductance K^+ channel is significantly higher than that of the voltage-sensitive K^+ channel (Table III). The activity of the hyperpolarization-activated K^+ channel was observed in 28 of 75 patches with GΩ seal, and the average NP_o is 0.40 ± 0.1. In contrast, the activity of

the low-conductance K^+ channel has been recorded in 39 out 75 patches with an average NP_o of 2.5 ± 0.3. In view of these differences, the hyperpolarization-activated K^+ channel does not appear to be the main type of channel responsible for maintaining the resting cell membrane potential. However, considering the large conductance of I.K. and the relatively high incidence of the channel, I.K. should contribute a significant part to the basolateral K^+ conductance. This notion is not fully supported by the observations made in the isolated CCD tubules, in which basolateral application of 10 mM TEA depolarized the membrane potential by only a moderate 2 mV (Schlatter, Lohrmann, and Greger, 1992), although the voltage-sensitive K^+ channel is blocked by 2 mM TEA applied to the internal surface of the cell membrane in the excised patches. It is conceivable that the discrepancy may result from a difference between the effects of internal and external application of the blocker.

Although the activity of the hyperpolarization-activated K^+ channel is probably not the predominant player in the basolateral K^+ conductance under control conditions, it could play an important role in the recycling of K^+ across the basolateral

TABLE II

Open- and Closed-Time Constants and Channel Open Probability in the Presence and Absence of PKA_c

	τ_o1	τ_o2	τ_c1	τ_c2	P_o	N
			ms			
$+PKA_c$	0.4 ± 0.08	8.2 ± 0.1*	0.3 ± 0.03	19.2 ± 0.1*	0.49 ± 0.03*	4
$-PKA_c$	0.4 ± 0.08	4.4 ± 0.1	0.3 ± 0.03	27.2 ± 0.2	0.12 ± 0.02	4

Results were obtained from four inside-out patches, and the pipette holding potential was 0 mV. PKA_c is the catalytic sununits of PKA (20 U/ml), and 100 μM MgATP was also present in the experiment with PKA_c. No MgATP was present in the experiment without PKA_c. Asterisks indicate values significantly different from the control value ($P < 0.01$).

membrane when Na^+/K^+-ATPase is stimulated by an increase in Na^+ influx. Under steady state conditions, K^+ ions entering the cell via the Na^+/K^+-ATPase are either secreted into the lumen or diffuse back to the basolateral side (recycling of K^+); thus, the intracellular K^+ concentration is maintained. However, when Na^+ entry is enhanced and transiently exceeds the rate of Na^+ extrusion as a consequence of an increase in Na^+ delivery or of stimulation of hormones, such as aldosterone or vasopressin, it leads to a transient increase in intracellular Na^+ concentration (Frindt, Silver, Windhager, and Palmer, 1993). As a consequence of the increase in intracellular Na^+ concentration, intracellular Ca^{2+} concentration increases (Frindt et al., 1993) and inhibits the Na^+ channel (Palmer and Frindt, 1987). This prevents further accumulation of intracellular Na^+ and cell swelling. On the other hand, the increase in Na^+ concentration stimulates the Na^+ pump, and consequently more K^+ is taken up into the cell. However, the increase in intracellular Ca^{2+} also blocks the activity of the apical K^+ channel (Wang, Geibel, and Giebisch, 1993). Thus, an augmentation in recycling of K^+ across the basolateral membrane is necessary to cope

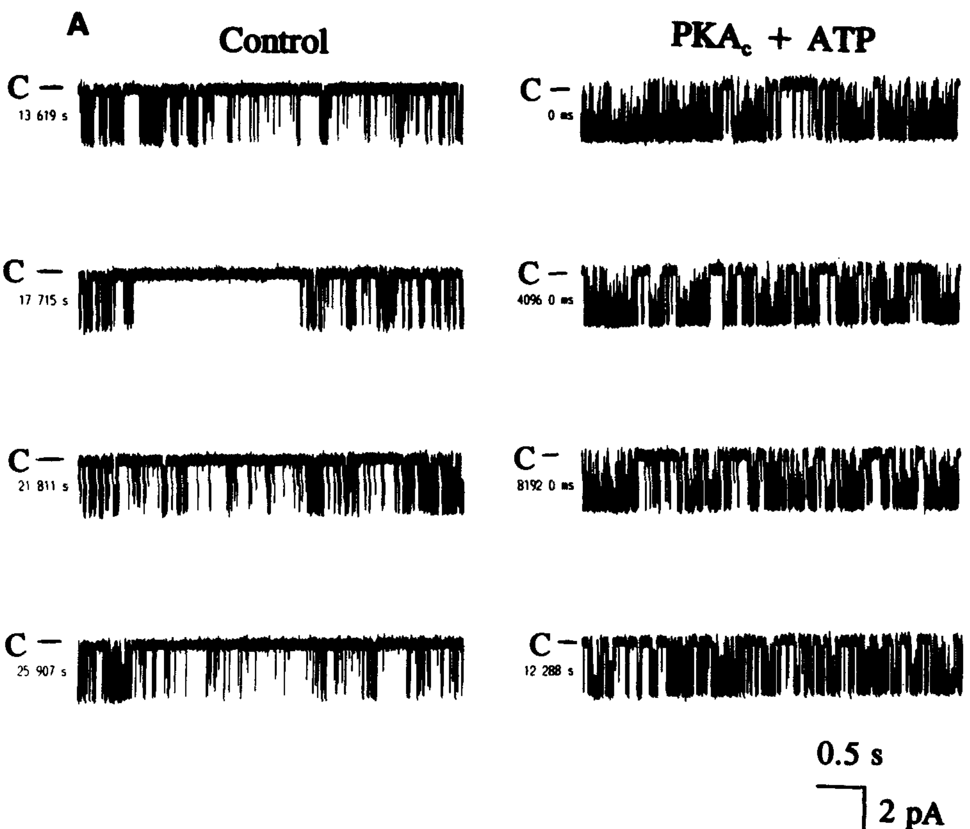

FIGURE 8. (*A*) A recording obtained from an inside-out patch with Ca^{2+}-free NaCl Ringer's solution in the bath under the control condition and in the presence of 100 mM ATP plus 20 U/ml PKA_c. The channel closed state is indicated by *C*, and the holding potential is 0 mV.

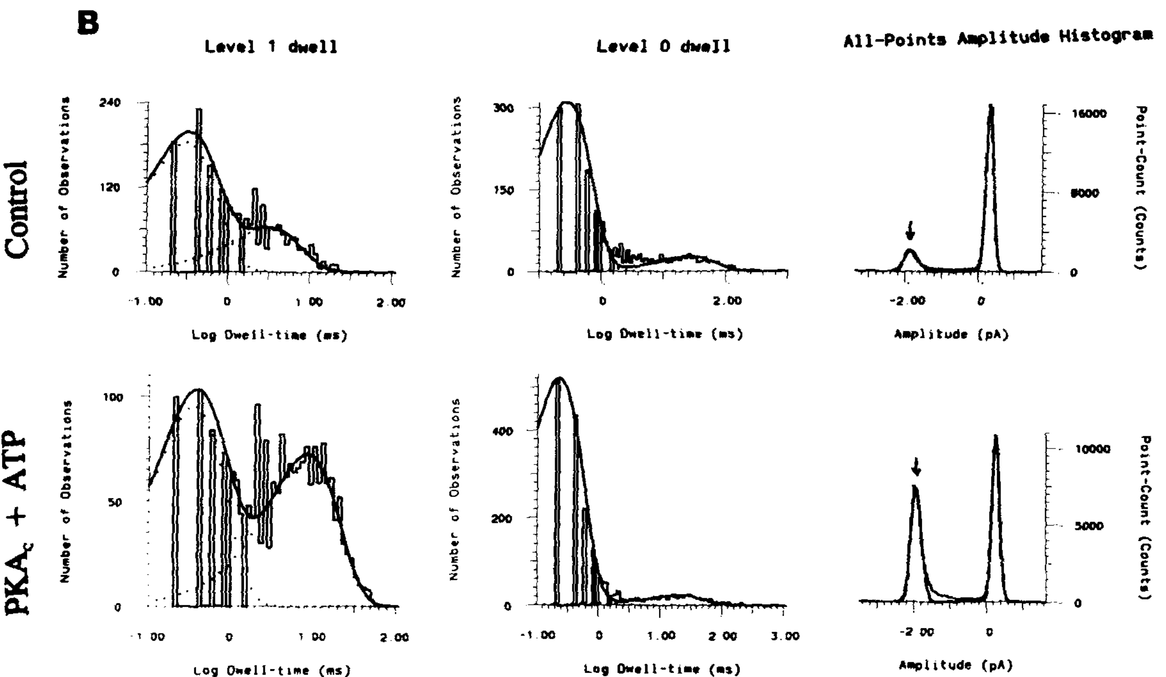

FIGURE 8. (*B*) The open-time and closed-time histograms and the all-points amplitude histograms under control condition (without PKA$_c$) and in the presence of PKA$_c$ plus 100 mM MgATP. (*Arrow*) Channel current in the open state.

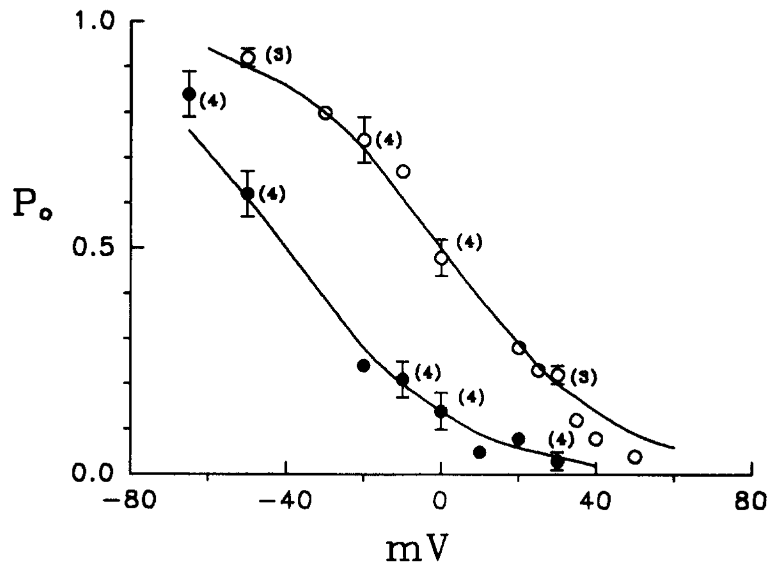

FIGURE 9. The voltage dependence curve of P_o fitted by the Boltzmann's equation described in the text (Eq. 3) in the absence of exogenous PKAc (*filled circle*) and in the presence of PKAc (*open circles*). The addition of PKAc shifts the midpoint ($V_{1/2}$) by 40 mV.

with K^+ ions taken up into the cell by the stimulated Na^+/K^+-ATPase. The activity of the intermediate-conductance K^+ channel can be stimulated by at least two mechanisms. First, hyperpolarization induced by an increase in the turnover rate of the Na^+/K^+-ATPase activates the channel activity. Second, hormones such as vasopressin stimulate adenylate cyclase and increases synthesis of cAMP (Breyer and Ando, 1994); PKA-induced channel phosphorylation then enhances the activity of the voltage-sensitive K^+ channel. Hyperpolarization activates the basolateral K^+ conductance in the isolated perfused amphibian collecting duct (Horisberger and Giebisch, 1988). Thus, this hyperpolarization-activated K^+ channel may serve an important role in the "pump–leak" mechanism for maintaining cell homeostasis (Schultz, 1981).

PKA-induced channel phosphorylation plays an important role in the modulation of a variety of ion channels (Sculptoreanu, Scheuer, and Catterall, 1993; Esguerra, Wang, Foster, Adelman, North, and Levitan, 1994; Huang, Morielli, and

TABLE III

Incidence and NP_o of the S.K. and I.K. in the Lateral Membrane in the CCD

	S.K.	I.K.
Number of seals	75	75
Number of patches with channels	39	28
Incidence	52%	37%
Mean NPo	2.5 ± 0.3 (N = 12)	0.4 ± 0.1 (N = 7)

The mean NP_o was calculated in the patches with one population of channel activity.

Peralta, 1994; Wilson and Kaczmarek, 1993; Wang, 1994). PKA-induced channel phosphorylation is essential for maintaining the activity of the apical low conductance K^+ channel in the CCD and TAL (Wang and Giebisch, 1991; Wang, 1994). In this study, we have shown that addition of PKA_c increases the activity of the intermediate-conductance K^+ channel. However, it is not clear whether PKA_c phosphorylates the K^+ channel protein or an associated protein, which in turn exerts the stimulatory effect on the K^+ channel. The main effect of PKA_c on channel activity is to shift the voltage dependence of channel activation to the positive side (depolarization). The shifts may be explained by the possibility that the PKA-induced phosphorylation may affect the stability of the open and the closed states of the channel.

The activity of the hyperpolarization-activated K^+ channel was pH sensitive: a decrease in bath pH from 7.4 to 6.7 led to a moderate reduction of channel activity by 30%. In contrast, the same range of pH changes had been shown to block completely the activity of the low-conductance K^+ channel in the apical membrane of the CCD. Interestingly, the activity of the low-conductance K^+ channel in the basolateral membrane was also much less sensitive to pH (Wang et al., 1994) than that in the apical membrane (Wang et al., 1990). Thus, alteration in cell pH had a more significant effect on K^+ conductance in the apical membrane than in the basolateral membrane. The different response to changes in cell pH between the apical and basolateral K^+ conductance may have an important physiological consequence during acidosis. It has been shown that acidosis significantly reduces K^+ secretion, whereas Na^+ reabsorption is less affected (Strieter, Weinstein, Giebisch, and Stephenson, 1992; Stanton and Giebisch, 1982). Thus, it is conceivable that the turnover rate of Na^+/K^+-ATPase is not significantly reduced by acidosis and, accordingly, the number of K^+ ions taken up by the Na^+ pump is not significantly reduced during acidosis. Because the apical K^+ conductance is compromised during acidosis, it is important to maintain the activity of the basolateral K^+ channels such that they are less affected than those in the apical membrane during acidosis, so as to provide a route for K^+ ions leaving the cell.

In conclusion, PKA-induced channel phosphorylation plays an important role in the modulation of the activity of the I.K. channel. This hyperpolarization-activated K^+ channel is not a main player in the basolateral K^+ conductance under basal conditions; however, it may play an important role in the rapid increase in the basolateral K^+ conductance in response to the stimulation of the activity of Na^+/K^+-ATPase.

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