

A conserved cytoplasmic region of ROMK modulates pH sensitivity, conductance, and gating

HAN CHOE, HAO ZHOU, LAWRENCE G. PALMER, AND HENRY SACKIN

Department of Physiology and Biophysics,

Cornell University Medical College, New York, New York 10021

Choe, Han, Hao Zhou, Lawrence G. Palmer, and Henry Sackin. A conserved cytoplasmic region of ROMK modulates pH sensitivity, conductance, and gating. *Am. J. Physiol.* 273 (*Renal Physiol.* 42): F516–F529, 1997.—ROMK channels play a key role in overall K balance by controlling K secretion across the apical membrane of mammalian cortical collecting tubule. In contrast to the family of strong inward rectifiers (IRKs), ROMK channels are markedly sensitive to intracellular pH. Using *Xenopus* oocytes, we have confirmed this pH sensitivity at both the single-channel and whole cell level. Reduction of oocyte pH from 6.8 to 6.4 (using a permeant acetate buffer) reduced channel open probability from 0.76 ± 0.02 to near zero ($n = 8$), without altering single-channel conductance. This was due to the appearance of a long-lived closed state at low internal pH. We have confirmed that a lysine residue (K61 on ROMK2; K80 on ROMK1), NH₂ terminal to the first putative transmembrane segment (M1), is primarily responsible for conferring a steep pH sensitivity to ROMK (B. Fakler, J. Schultz, J. Yang, U. Schulte, U. Brãandle, H. P. Zenner, L. Y. Jan, and J. P. Ruppersberg. *EMBO J.* 15: 4093–4099, 1996). However, the apparent pK_a of ROMK also depends on another residue in a highly conserved, mildly hydrophobic area: T51 on ROMK2 (T70 on ROMK1). Replacing this neutral threonine (T51) with a negatively charged glutamate shifted the apparent pK_a for inward conductance from 6.5 ± 0.01 ($n = 8$, wild type) to 7.0 ± 0.02 ($n = 5$, T51E). On the other hand, replacing T51 with a positively charged lysine shifted the apparent pK_a in the opposite direction, from 6.5 ± 0.01 ($n = 8$, wild type) to 6.0 ± 0.02 ($n = 9$, T51K). The opposite effects of the glutamate and lysine substitutions at *position 51* (ROMK2) are consistent with a model in which T51 is physically close to K61 and alters either the local pH or the apparent pK_a via an electrostatic mechanism. In addition to its effects on pH sensitivity, the mutation T51E also decreased single-channel conductance from 34.0 ± 1.0 pS ($n = 8$, wild type) to 17.4 ± 1 pS ($n = 9$, T51E), reversed the voltage gating of the channel, and significantly increased open-channel noise. These effects on single-channel currents suggest that the T51 residue, located in a mildly hydrophobic area of ROMK2, also interacts with the hydrophobic region of the permeation pathway. potassium; channel; inward rectifier; kidney; pK_a

POTASSIUM SECRETION in the mammalian cortical collecting tubule (CCT) is primarily controlled by a mildly inward rectifying, potassium channel (SK), located at the apical membrane of CCT principal cells (5, 15). Expression cloning resulted in the putative identification of the SK channel with the ROMK family of mild inward rectifiers, consisting of the following three splice variants: ROMK1 (K_{ir}1.1a; see Ref. 8), ROMK2 (K_{ir}1.1b; see Ref. 18), and ROMK3 (K_{ir}1.1c; see Ref. 1). ROMK2 lacks the first 19 amino acids of ROMK1, whereas ROMK3 contains a 7-amino acid extension.

Potassium channels in the inward rectifier superfamily (ROMK, IRK, GIRK) are thought to consist of four identical subunits surrounding a central pore (7, 17). Hydrophathy analysis of the primary structure of these subunits suggests a common motif, consisting of two putative membrane spanning domains (M1 and M2) separated by a relatively short loop thought to be associated with the permeation path or pore region. Both the COOH-terminal and NH₂-terminal segments of the subunits are devoid of long hydrophobic stretches and are presumed to be cytoplasmic.

Despite similarities in structure among members of the IRK superfamily, the ROMK channels display a much greater sensitivity to intracellular pH (pH_i) than do the strong inward rectifiers like IRK1 (4). Reductions in pH_i from 7.4 to 6.8 greatly decrease the open probability (P_o) of native SK channels (15), as well as ROMK channels expressed in *Xenopus* oocytes (10, 11, 14). This marked sensitivity of the ROMK family to pH_i may be important for K homeostasis during different metabolic states. Specifically, the clinical observation that metabolic alkalosis is often accompanied by enhanced K secretion and hypokalemia could partly be explained by augmentation of luminal CCT potassium permeability by increases in pH_i (13, 16).

MATERIALS AND METHODS

Clones. The primary structure of the ROMK2 clone (GenBank accession no. L29403) has been previously described (18). The clone was obtained from a cDNA library made from rat kidney poly(A)⁺ RNA in the plasmid vector pSPORT. To obtain RNA from the clone, plasmid DNA was purified with the Qiagen anion-exchange column system (Qiagen, Chatsworth, CA). Plasmid DNA was linearized with *Not* I and transcribed in vitro with T7 RNA polymerase in the presence of the GpppG cap using mMESSAGING mMACHINE kit (Ambion, Austin, TX). Synthetic RNA was dissolved in water and stored at -70°C before use. Mutations were made using the overlap extension method (9). Nucleotide sequences were checked using the dideoxy chain termination method with a Sequenase kit (US Biochemical, Cleveland, OH).

Oocytes. Stage V-VI oocytes were obtained by partial ovariectomy of female *Xenopus laevis* (Xenopus-I, Ann Arbor, MI), anesthetized with tricaine methanesulfonate (1.5 g/l, adjusted to pH 7.0). Oocytes used for the whole cell, two-electrode voltage clamp (TEVC) experiments were defolliculated by incubation with 2 mg/ml collagenase type II and 2 mg/ml hyaluronidase type II (Sigma Chemical, St. Louis, MO) for 60 min at 23°C and stored overnight at 19°C in modified Leibovitz L-15 medium (18). On the next day, healthy oocytes were selected for RNA injection.

Oocytes destined for patch-clamp experiments were defolliculated by incubation with 4 mg/ml collagenase type II and 4 mg/ml hyaluronidase type II (Sigma Chemical) for 60 min at 23°C and then exposed to hypertonic media (460 mosmol/

kgH₂O) for 15 min. Only those oocytes exhibiting a clear separation between vitelline and plasma membranes were selected, returned to solutions of normal osmolarity, and saved overnight at 19°C in Leibovitz L-15 medium (2, 3). This modification in the oocyte preparative technique significantly reduced adhesions between the vitelline and plasma membranes and greatly enhanced the probability of forming high-resistance seals at the time of patching (3). Oocytes used for either TEVC or patch-clamp were injected with 0.5 to 1 ng cRNA and incubated at 19°C in Barth's solution supplemented with Leibovitz medium for 1–3 days before measurements were made.

Electrical. Whole oocyte current-voltage (*I-V*) relationships were obtained, in intact oocytes, using a TEVC with 3 M KCl-filled current and voltage electrodes, as previously described (2). Currents were recorded for 50 ms at each voltage, using a pulse protocol in which membrane potential (V_m) was stepped by 10 mV from +60 mV to -140 mV, interspersed with a return to the resting V_m . The oocytes reserved for single-channel measurements were again subjected to a hypertonic shrinking solution, thereby allowing the vitelline membrane to be easily removed. Single channels were studied using the patch-clamp technique as previously described (2, 18).

The stripped oocytes were placed in a small Lucite chamber where the bath solution could be exchanged by gravity perfusion. All measurements were made at room temperature. Methods for data acquisition and analysis have been described in detail elsewhere (6, 12). Pipettes were pulled from hematocrit tubing on a three-stage puller and were coated with Sylgard prior to use. Currents were recorded with either List EPC-7 or Dagan 8900 patch-clamp amplifiers and stored, unfiltered, on videotape. For analysis, current records were replayed from videotape, sampled at 5 kHz, low-pass filtered at 1 kHz, and analyzed using an Atari-based data acquisition system and TAC software (Instrutech, Mineola, NY).

Solutions. In the TEVC experiments, initial measurements of resting potential were performed with (in mM) 105 NaCl, 5 KCl, 1 MgCl₂, 2 CaCl₂, and 5 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), adjusted to pH 7.4 with NaOH. Those experiments examining the effects of internal oocyte pH on macroscopic conductance utilized high-K bath solutions consisting of (in mM) 55 KCl, 55 potassium acetate, 1 MgCl₂, and 2 CaCl₂, buffered with 10 HEPES, and adjusted to a final pH between 6.3 and 8.2 with KOH. As previously described (14), internal oocyte pH could be controlled with membrane-permeable potassium acetate solutions. However, this method of changing oocyte pH required about a 20-min equilibration period for each new pH, and the measured internal pH was always significantly lower than bath pH (see below).

In the patch-clamp experiments, oocytes were bathed in a high-K bath solution, consisting of either 110 mM KCl or 55 mM KCl + 55 mM potassium acetate (for the pH studies) and 2 mM CaCl₂, buffered with 10 HEPES. Pipettes were filled with either 110 mM KCl or 55 mM KCl + 55 mM potassium acetate (for the pH studies) and 1 mM MgCl₂, buffered with 10 mM HEPES. In the patch experiments, MgCl₂ was omitted from the bath solution, because it accelerates rundown in excised patches, but it was retained in the pipette solution, because the single-channel conductance of ROMK is Mg²⁺ dependent. All other chemicals were obtained from Sigma Chemical.

Intracellular pH measurements. pH_i was measured with pH-selective microelectrodes in a small sample of oocytes to determine how well the membrane-permeable acetate-buffered solutions controlled pH_i. Glass capillaries (model GC

200 F-10; Warner Instruments, Hamden, CT) were pulled on a horizontal microelectrode puller (model P-97; Sutter Instruments, San Francisco, CA). pH-selective electrodes were silanized by exposure to 40 μl of bis(dimethylamino)-dimethylsilane (catalog no. 14755, Fluka Chemical) in a covered glass container at 200°C, and the tips were coated with hardened Sylgard (Dow Corning) to reduce electrical noise. The tip of the pH electrode was filled (from the back) with Hydrogen ionophore I-Cocktail B (catalog no. 95293, Fluka) and then back-filled with pH 7 solution of 0.04 M KH₂PO₄, 0.023 M NaOH, and 0.015 M NaCl and inserted into an Ag/AgCl half-cell electrode holder. The pH electrodes had resistances of ~5 × 10¹¹ Ω and were calibrated using commercial standard pH 6 and pH 8 solutions. The mean slope of the electrodes used in this study was 56.2 ± 0.6 mV/pH unit. Voltage electrodes were pulled as above but filled with 3 M KCl and had tip resistances of 30–60 MΩ. The voltage due to pH_i was obtained by electronically subtracting the potential recorded by the voltage microelectrode from the voltage recorded by the pH microelectrode using a high-impedance electrometer (model FD 223; World Precision Instruments, Sarasota, FL). Intracellular voltage and pH were continuously displayed with an online computer system. The pH_i measurements reported in this study were conducted at Yale Univ. Dept. of Physiology in the laboratory of Dr. W. F. Boron, with the expert assistance of Dr. G. Cooper and Dr. M. Romero.

RESULTS

Control of oocyte pH with acetate solutions. To determine the time course and effectiveness of membrane-permeable, acetate-buffered solutions at controlling oocyte pH, pH-selective (resin-filled) microelectrodes were used to measure pH_i in a small group of oocytes having either high or low K conductance (see MATERIALS AND METHODS). When initially placed in a 2 mM K-Ringer solution, those oocytes expressing large amounts of wild-type ROMK2 (WT-ROMK2) channels for several days had generally more negative resting potentials (-90 ± 5 mV) and slightly lower initial pH_i (7.13 ± 0.03) than low K conductance, water-injected oocytes (-73 ± 6 mV, initial pH_i = 7.32 ± 0.02).

Immersion in 55 mM KCl + 55 mM potassium acetate depolarized both high and low K conductance oocytes to resting potentials near zero mV. Progressive exposure of these oocytes to potassium acetate-chloride solutions buffered at pH values between 8.2 and 6.3 decreased oocyte pH_i from 7.2 to 6.1 where steady-state pH_i values were generally obtained at 20–25 min after a change in external pH. These results are summarized in Table 1 for the five bath pH values tested on either ROMK2- or water-injected oocytes from three frogs.

In all oocytes, steady-state pH_i was always lower than the bath pH, although the degree of deviation depended on extracellular pH. Furthermore, exposure to acetate solutions always decreased pH_i below its resting value, as initially measured in 2 mM K Ringer solution. It was not possible to alkalinize the oocytes with extracellular potassium acetate solutions.

Effect of pH on single-channel ROMK2 currents. Results of the present study confirm the pH sensitivity of ROMK at both the single channel and whole cell level. Expression of ROMK2 in *Xenopus* oocytes yielded

Table 1. Control of intracellular oocyte pH using a membrane-permeable acetate buffer

Bath pH	pH _i	No. of oocytes
8.2	7.2	5
7.8	7.0	5
7.4	6.8	5
6.8	6.4	5
6.3	6.1	5

Data were obtained either with oocytes expressing wild-type ROMK2 channels or with water-injected oocytes. Since the results of the 2 groups were similar, the data were pooled. All oocytes were depolarized in high-K solutions: 55 mM potassium acetate, 55 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂ and HEPES. Bath pH was adjusted with KOH. Intracellular pH (pH_i) was continuously monitored with pH-selective electrodes (see MATERIALS AND METHODS). Steady-state values reported for pH_i were obtained between 20 and 25 min after a change in bath pH.

channels with high open probability and mild inward rectification as previously described (2). In cell-attached patches with one channel in the patch, intracellular acidification (via permeant acetate buffers) consistently reduced P_o .

A complete time course illustrating the effect of lowering pH_i on ROMK2 is given in Fig. 1. The cell-attached patch contained only one channel, and the oocyte was maintained at -100 mV relative to the pipette throughout the 20-min recording. In this experiment, extracellular pH was changed from 7.4 to 6.8 using the membrane-permeable acetate buffer described (MATERIALS AND METHODS). According to the pH_i measurements (Table 1), this would correspond to a steady-state change in oocyte pH_i from 6.8 to 6.4. Although pH_i was not directly measured in this particular experiment, the decline in pH_i roughly paralleled the decrease in channel activity. Comparison of the time course of pH_i and inward current is described below. About 10 min after the change in bath pH from 7.4 to 6.8, there was a noticeable increase in long closures characterized by the appearance of a third closed state in the kinetic scheme (Fig. 2B). This effect could be reversed by returning the bath pH to 7.4. In five single-channel recordings (each on a separate oocyte), the average lifetime of this third long-closed state was 2.6 ± 0.5 s (Table 2).

In five experiments at a bath pH of 7.4 and a V_m of -100 mV, the average open probability for WT-ROMK2 was $P_o = 0.76 \pm 0.02$. Reduction of bath pH to 6.8 decreased P_o in all five cases. Analysis of cell-attached current records 30 s preceding shutdown of the channel yielded the kinetic data shown in Table 2, *middle*. This decrease in open probability to $P_o = 0.38 \pm 0.11$ ($n = 5$) could be completely explained by the appearance of a very long closed state (Fig. 2B). In five recordings, return to a bath pH of 7.4 (steady-state pH_i = 6.8) caused a disappearance of the this long closed state and restored the open probability to $P_o = 0.72 \pm 0.06$.

As indicated by the example of Figs. 1 and 2, the period of long closures persisted for ~ 20 – 30 s until a complete closure occurred, producing an apparent cessation of channel activity. It was possible to restore

channel activity if bath pH were returned to 7.4 within about 45 s of channel shutdown. Allowing channels to remain for more than 45 s in the closed state at low pH_i prevented reactivation upon return to bath pH 7.4. In addition, the long closures prior to complete shutdown were often preceded by the random appearance of discrete substates (circled events in Fig. 1). These also appeared at the beginning of channel recovery following return to a bath pH of 7.4. These substates were too few in number to study, and their significance is not understood.

The pH-dependent shutdown of the channel did not alter the lifetime of either the open state or the two shorter closed states. This is illustrated in Fig. 2, and the kinetic data are summarized in Table 2. Single-channel conductance also remained the same during the pH-dependent shutdown of the channel (Fig. 2D). The decrease in P_o at low pH can be attributed to the appearance of a long closed state.

Channels in excised (inside-out) patches had a similar, but somewhat faster, response to alterations in cytoplasmic-side pH (Fig. 3) than was observed with cell-attached patches. ROMK2 channel activity in the excised patch of Fig. 3 was completely blocked by a bath pH of 6.4, although this required 47 s after the start of the bath solution change. Since bath exchange was complete within 10 s, pH shutdown of the channel is not immediate, even in excised patches. Furthermore, channel activity in the excised patch did not recover until more than a minute after return of the bath pH to 7.4 (Fig. 3).

Similar to the results with cell-attached patches, a complete absence of channel activity for more than 45 s at low pH prevented recovery, even after a return of bath pH to 7.4. In no case did a reduction in pH cause significant changes in current amplitude, although the rundown that often occurred with excised patches sometimes reduced current amplitude before complete disappearance of channel activity. The reason for this is not known, but, to avoid confusion, average kinetic parameters were computed only for cell-attached patches.

pH_i dependence of inward rectifier macroscopic currents. Macroscopic whole cell currents from WT-ROMK display a marked sensitivity to pH_i (14), analogous to the pH sensitivity of the native small K channel in native rat CCT (15). Our studies confirmed this steep dependence of inward conductance on pH_i (solid line of Fig. 4). For WT-ROMK2 the average pK_a was 6.5 ± 0.01 (8 oocytes), and the Hill coefficient was 5.2 ± 0.6 , indicating that permeation through this channel is turned on and off over a relatively narrow range of pH.

In the experiments used to construct Fig. 4, inward conductances were measured at different pH values and normalized to the maximum inward conductance for each oocyte. Intracellular pH was controlled with extracellular potassium acetate solutions, where stepwise decreases in extracellular pH from 8.2 to 6.3 reduced pH_i from 7.2 to 6.1 (see MATERIALS AND METHODS Table 1).

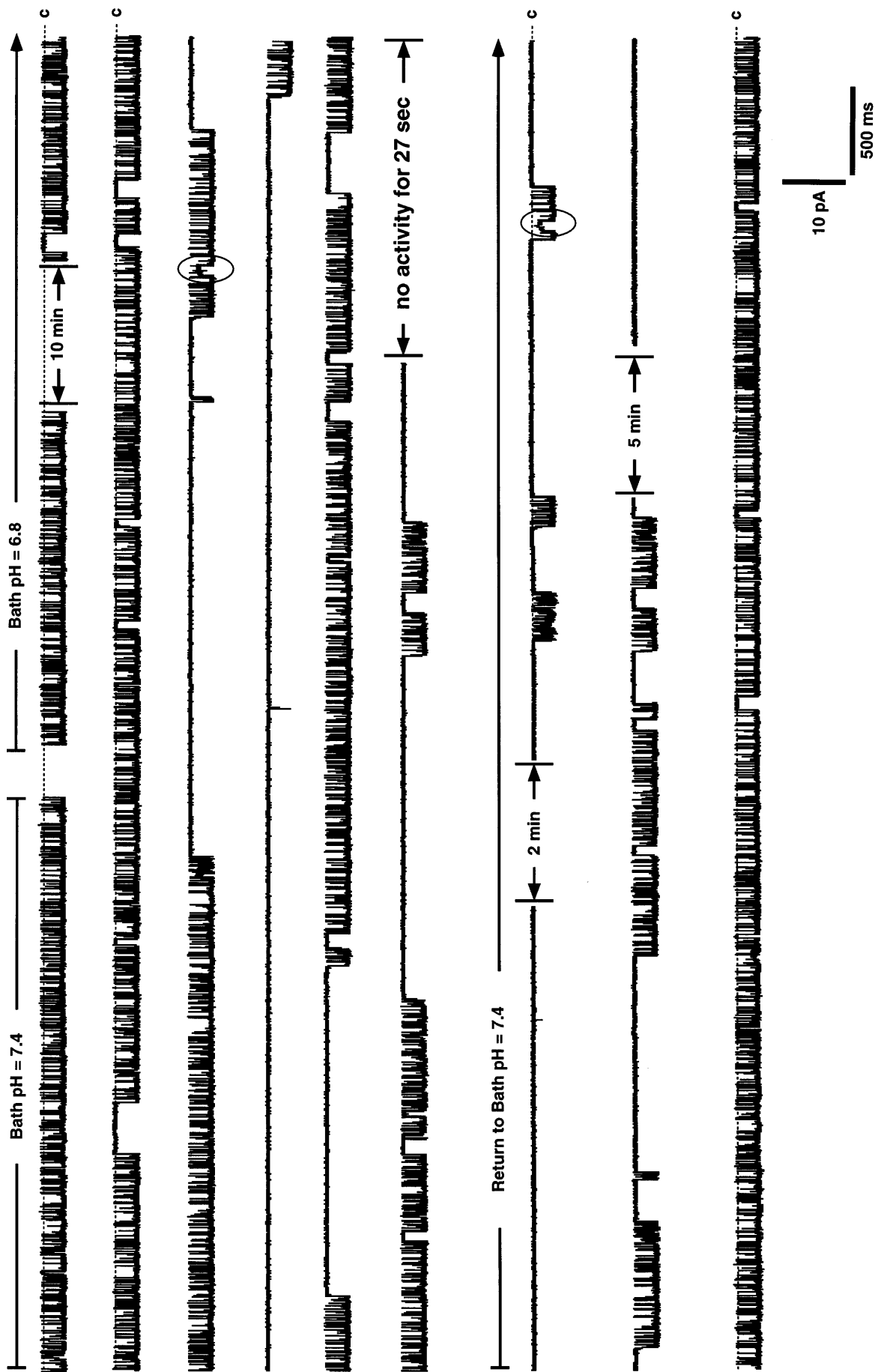


Fig. 1. Effect of changing internal pH from 7.4 to 6.8 on wild-type ROMK2 (WT-ROMK2) currents recorded from a single channel in a cell-attached patch on an oocyte depolarized by 55 mM KCl + 55 mM potassium acetate. Oocyte was maintained at -100 mV relative to pipette throughout the changes in internal pH, which were accomplished with a membrane-permeable acetate buffer. Bath pH values of 7.4 and 6.8 correspond, respectively, to intracellular pH (pH_i) values of 6.8 and 6.4. Downward deflections from the closed state (dotted line) denote inward currents. Circled events denote subconductances, appearing before channel shutdown and shortly after reactivation.

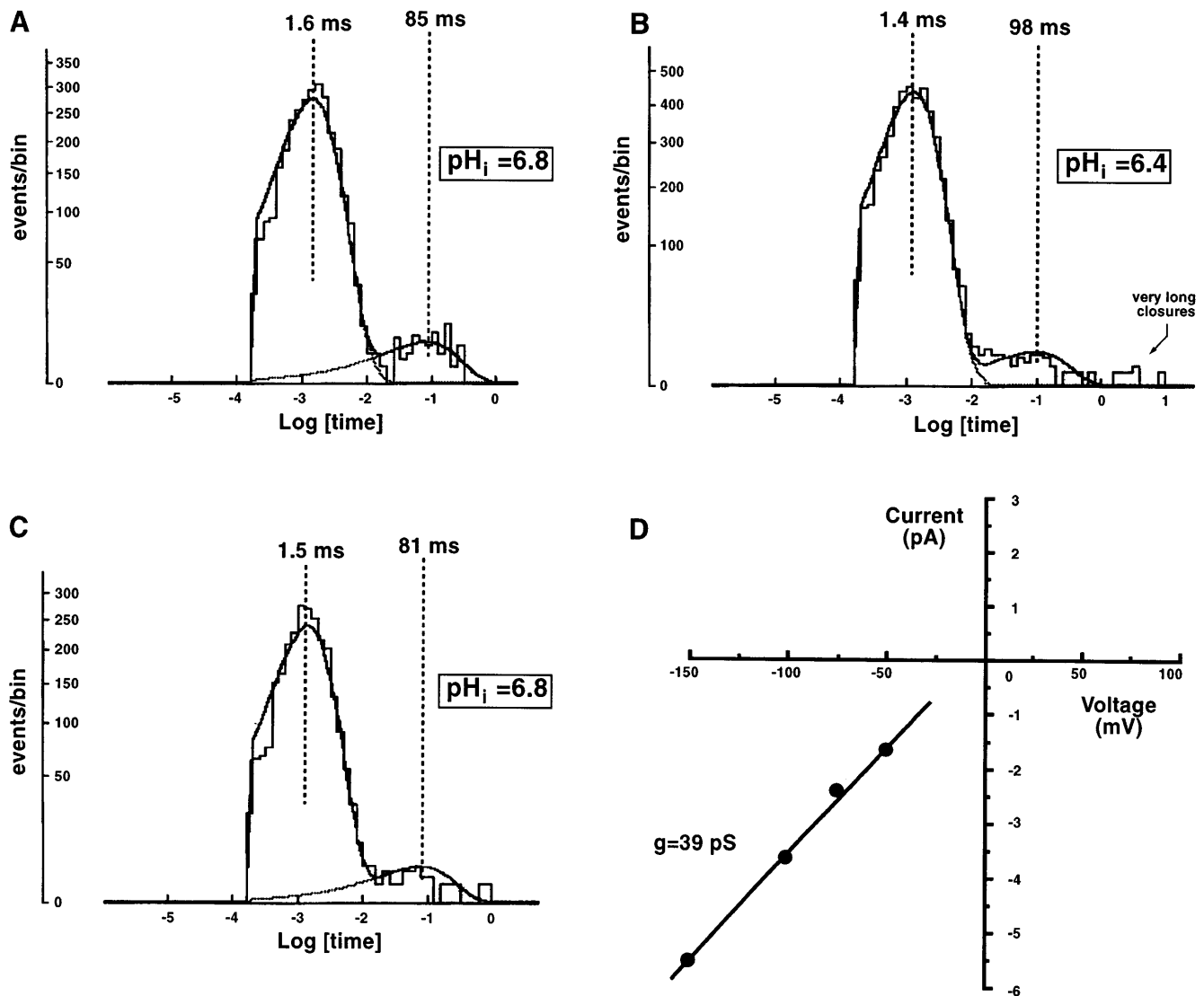


Fig. 2. Effect of decreasing pH_i on closed-time histograms of WT-ROMK: analysis of cell-attached patch, containing a single channel (records shown in Fig. 1). Oocyte was maintained at -100 mV relative to pipette throughout the changes in internal pH, which were accomplished with a permeant buffer. *A*: closed-time distribution for initial recording at a bath pH of 7.4 ($pH_i = 6.8$), showing 2 discrete closed states with time constants of 1.6 ms and 85 ms and open probability (P_o) = 0.8. *B*: 6 min after changing external, acetate-buffered solution to pH 6.8 ($pH_i = 6.4$). Note appearance of very long closed state; $P_o = 0.2$. *C*: recovery of channel activity 3 min after return of bath to pH 7.4 ($pH_i = 6.8$), showing 2 closed states with time constants of 1.5 ms and 81 ms. Note disappearance of very long closed state. *D*: single-channel conductance (39 pS in this experiment) was unaffected by changes in pH.

The steep dependence of ROMK2 currents on pH_i was not shared by other members of the K_{ir} family (IRK1), which were essentially insensitive to pH_i between 7.2 and 6.2. As indicated in Fig. 4, the strong inward rectifier (IRK1) exhibited nearly constant inward conductance until pH_i dropped below 6.1. Conductance block at this pH_i may involve a completely different process whereby hydrogen ions directly interfere with the permeation path (4).

Although internal oocyte pH was not measured during the TEVC experiments of Fig. 4, the time course for the change in oocyte pH_i was determined in a separate batch of oocytes from several frogs (see MATERIALS AND METHODS). The results are illustrated in Fig. 5, which

compares the time course of oocyte pH_i and inward current during a decrease in bath pH from 7.4 to 6.8 (corresponding to a change in steady-state pH_i from 6.8 to 6.4). The squares (and solid line) of Fig. 5 depict inward current, measured at an oocyte potential of -60 mV and normalized to its maximum value at *time 0*. The triangles (dashed line) in Fig. 5 represent the oocyte pH measured with pH-selective microelectrodes. As shown in Fig. 5, the average time course for the reduction in pH_i was slightly longer (7.6 ± 0.6 min $^{-1}$) than the time course for reduction of inward current (5.6 ± 0.2 min $^{-1}$). In any case, macroscopic oocyte currents were routinely measured 25–30 min after a change in bath solution. At these times the

Table 2. *pH dependence of single-channel kinetic parameters for wild-type ROMK2*

	Bath pH = 7.4	Bath pH = 6.8	Bath pH = 7.4
pH_i	6.8 ± 0.04	6.4 ± 0.04	6.8 ± 0.04
P_o	0.76 ± 0.02	$0.38 \pm 0.11^*$	0.72 ± 0.06
τ_{open} , ms	13.6 ± 0.8	12.2 ± 1.0	11.4 ± 0.7
τ_1^{closed} , ms	1.6 ± 0.2	1.5 ± 0.2	1.5 ± 0.2
τ_2^{closed} , ms	89 ± 10	101 ± 10	79 ± 4
τ_3^{closed} , ms	NO	$2,640 \pm 530$	NO

Data are from 5 oocytes, recorded in the cell-attached mode from patches containing a single channel, recorded at a membrane potential (V_m) = -100 mV. Bath consisted of membrane-permeable acetate-buffered solutions, titrated to either pH 7.4 or pH 6.8. pH_i values refer to the oocyte pH, as determined from microelectrode measurements (see Table 1). P_o , single channel open probability. Kinetic parameters: τ_{open} , open time; τ_1^{closed} , shorter closed-time constant; τ_2^{closed} , intermediate closed-time constant; and τ_3^{closed} , closed-time constant for very long closures. *Significantly different ($P < 0.05$) from values measured at the initial $pH_i = 6.8$. Third closed state not observed (NO) at $pH_i = 6.8$. Values in *middle* column (Bath pH = 6.8) were obtained during the 30 s immediately preceding complete shutdown of the channel.

currents, and presumably pH_i , should both be in a steady state.

Recently, it was discovered that a single lysine (K61 on ROMK2, K80 on ROMK1), NH_2 terminal to the first hydrophobic segment (M1), is essential for conferring steep pH sensitivity to ROMK (4). This result was confirmed in the present study in which replacing the lysine at *position 61* with methionine (K61M) abolished the pH sensitivity of ROMK2 (K61M mutant in Fig. 4).

pH_i dependence of T51 mutant macroscopic currents. Both IRK and ROMK channels possess a highly conserved, mildly hydrophobic area (designated the "Q" region) that is located immediately NH_2 terminal to the first transmembrane segment (M1) of both inward rectifiers (Fig. 6). The "Q regions" of the inward rectifier superfamily show a high degree of homology. The boldface residues of Fig. 7 indicate amino acids that are identical to those of ROMK2. A cursory inspection of Fig. 7 reveals that many of the nonidentical residues in the same column have similar properties. Finally, the hydrophobicity and proximity of the Q region to the M1 transmembrane segment raises the possibility that this region might be involved in the permeation process.

Two completely conserved residues of the Q region are the pair of threonines at *positions 51* and *52* (indicated by the rectangle in Fig. 7). Hence, we chose to examine the effect of mutations at this site on the conduction properties of ROMK2. As shown below, this did indeed produce alterations in the K conductance of the channel. Unexpectedly, the normally permeant cation NH_4 blocked the mutant channels. Since extracellular NH_4 is known to reduce pH_i in the oocyte, we tested whether this phenomenon could be explained by an enhanced pH sensitivity of the channel.

As illustrated in Fig. 8 (and Table 3), substitution of glutamate for threonine at *position 51* shifted the apparent pK_a for inward conductance from 6.5 ± 0.01 for WT-ROMK2 to 7.0 ± 0.02 for T51E. The analogous

substitution at position T52 had no significant effect on the apparent pK_a (T52E, dotted line, Fig. 8). In contrast, the introduction of a positively charged lysine at location T51 shifted the apparent pK_a in the opposite direction to 6.0 ± 0.02 (T51K, Fig. 8 and Table 3).

The curves in Figs. 4 and 8 were constructed by fitting the data to the Hill equation for normalized inward conductance: $G/G_{max} = 1/[1 + ([H]/K)^n]$, where [H] is the internal hydrogen ion concentration, n is the Hill coefficient, and $pK_a = -\log_{10}K$. Results of the parameter fit are summarized in Table 3. The Hill coefficient for WT-ROMK was $n = 5.2 \pm 0.6$, indicating that the process underlying the pH regulation is highly cooperative. The mutants T51E and T51K had significantly lower Hill coefficients (Table 3). No acceptable fit to the Hill equation could be obtained for K61M or IRK1.

We also examined whether the greater sensitivity to protons of T51E could still be observed in the absence of the critical lysine (K61). Evaluation of the double mutant (K61M/T51E) indicated that it had no pH sensitivity in the range between 6 and 8, similar to the result obtained with K61M alone and IRK1 (Fig. 4). This suggests that K61 is essential for the pH sensitivity of ROMK2, whereas the (charge of the) residue at *position 51* modulates this pH sensitivity and helps to determine the pK_a of the channel.

Single-channel properties of T51E. The "P" region (Fig. 6) is a hydrophobic stretch of amino acids that are thought to form at least part of the pore in both voltage-gated and inward rectifier K channels. The similarity of the Q and P regions with regard to hydrophobicity and proximity to the transmembrane domain raises the possibility that the Q region also contributes to the formation of the pore. This was assessed by comparing single-channel records from T51E mutants and WT-ROMK2 channels. Figure 9A illustrates a typical cell-attached recording of inward K currents through T51E. Both bath and pipette contained 110 mM KCl, and the average resting potential of the oocytes was about zero mV, implying an oocyte K concentration close to that of the bath. To maximize the signal-to-noise ratio for T51E, the voltage clamp was set so that the oocyte interior was effectively -200 mV relative to the pipette. Downward deflections from the closed state (dotted line, Fig. 9A) correspond to K current from pipette to oocyte. The single-channel conductance in the inward direction for this experiment was 16 pS, as determined from currents at voltages between 0 and -200 mV.

The T51E records of Fig. 9 can be contrasted with those from WT-ROMK2, which exhibited low open-channel noise and an inward conductance of 33 pS (Fig. 10). Although both Figs. 9 and 10 depict current records with the oocyte clamped at -200 mV relative to pipette, qualitatively similar results were obtained at more physiological V_m values of -80 mV (oocyte relative to pipette). At all voltages and over a pH_i range from 6.1 to 7.2, T51E channels consistently exhibited smaller current amplitudes and greater open-channel noise than wild-type currents.

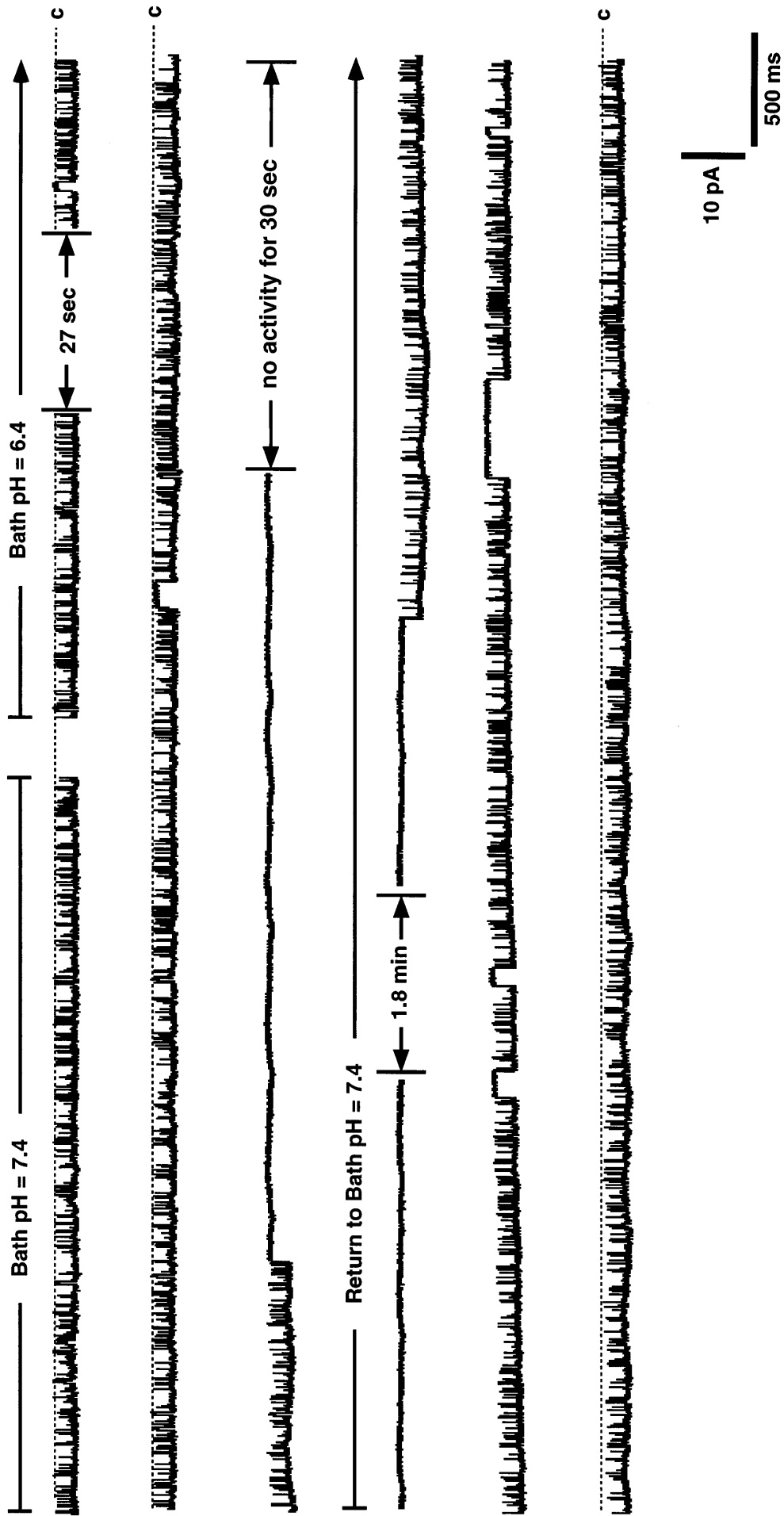


Fig. 3. Effect of changing cytosolic side pH from 7.4 to 6.4 on WT-ROMK2 currents recorded from a single channel in an excised (inside-out) patch in a high-K bath (55 mM KCl + 55 mM potassium acetate). Cyttoplasmic side of the membrane was maintained at -100 mV relative to pipette throughout the changes in cytoplasmic-side pH. Downward deflections from the closed state (dotted line) denote inward currents.

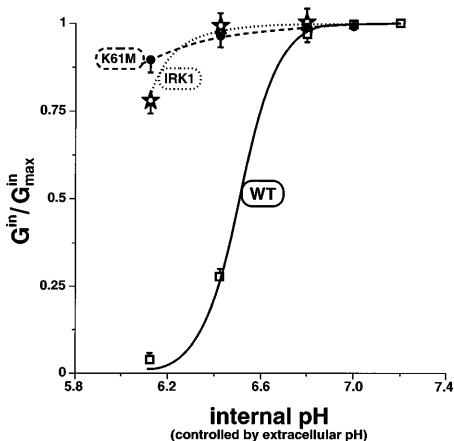


Fig. 4. pH dependence of WT-ROMK2, IRK1, and K61M mutants. Ordinate: normalized inward conductance (at -80 mV) of oocytes expressing either WT-ROMK2 (\square , solid line), IRK1 (\star , dotted line) or K61M (\bullet , dashed line). Abscissa: pH_i controlled with extracellular potassium acetate solutions (see text). Conductances at different values of internal pH were normalized to the maximum inward conductance for that oocyte. Data were fit to a modified Hill equation (see text).

Since the current records in Figs. 9 and 10 were obtained from single-channel patches, the kinetics of T51E and WT-ROMK2 could be readily compared. Figure 9, B and C, vs. Fig. 10, B and C, indicates that T51E (at $V_m = -200$ mV) has a somewhat greater long closed time than wild type (25 vs. 17 ms), but this is

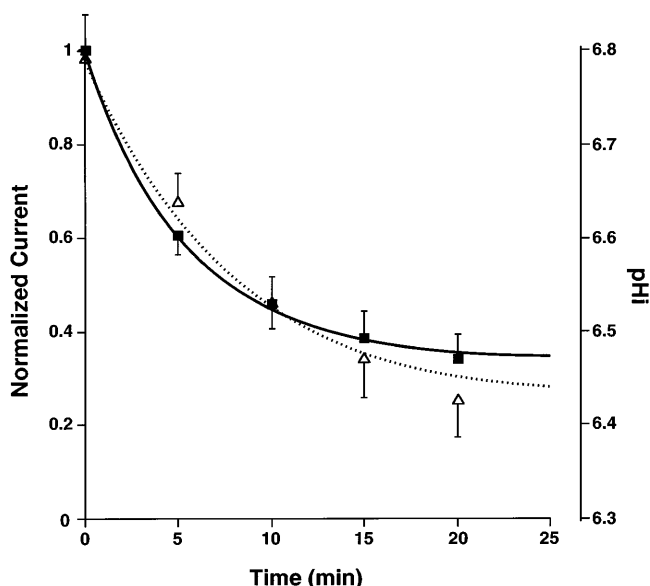


Fig. 5. Comparison of macroscopic current and intracellular pH (pH_i) time course for WT-ROMK2. For both curves the bath pH was changed from 7.4 to 6.8 using potassium acetate solutions. This produced a decline in steady-state pH_i from 6.8 to 6.4 in 20 min. Solid line (\blacksquare) are the measured normalized macroscopic inward currents at -60 mV during the change in bath pH. This was fit to a single exponential with a time constant of 5.6 ± 0.2 min $^{-1}$. Dashed line (\triangle), time course for pH_i during this same period. This was fit to a single exponential with a time constant of 7.6 ± 0.6 min $^{-1}$.

offset by a longer open time (5.2 vs. 4.8 ms). The combined effect leaves the open probability similar for mutant ($P_o = 0.72$) and wild type ($P_o = 0.74$) at V_m values of -200 mV. In contrast (discussed below), the P_o of T51E decreases dramatically with depolarization, so that at normal cell potentials of -70 mV, T51E has a much lower P_o than WT-ROMK2.

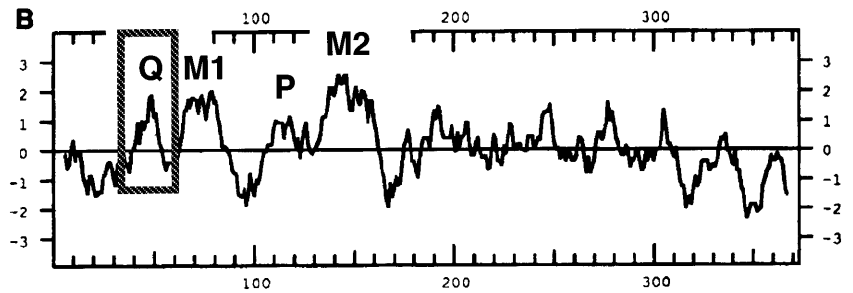
Single-channel $I-V$ relations (Fig. 11) were constructed from cell-attached current records at different holding potentials. In all cases, the patch pipette contained 1 mM $MgCl_2$ + 110 mM KCl, and the bath was maintained at 110 mM KCl, which depolarized the oocyte resting potential to zero. Both wild type and T51E mutants showed mild inward rectification at the single channel level, characteristic of ROMK channels. Consistent with the current records of Figs. 9 and 10, the average inward single-channel conductance of T51E was 17 ± 1 pS in eight oocytes, about one-half the single-channel conductance of WT-ROMK2 (34.8 ± 0.5 pS in 5 oocytes).

If the wild type and mutant current amplitudes are normalized by their respective inward conductances, then the single-channel $I-V$ relations are practically superimposable (Fig. 12). When viewed in this way, there is no difference in single-channel rectification between wild type and the T51E mutant. This is in contrast to the normalized macroscopic currents in which T51E displayed 40% more rectification than WT-ROMK (Fig. 13). In Fig. 13, all currents were normalized to the maximum inward current at -80 mV. Similar results were obtained at bath pH values of 7.4 and 7.8. When 10 mM K rather than 110 mM K was used in the bath, reversal potentials of the macroscopic $I-V$ relations indicated no difference in ion selectivity between WT-ROMK and T51E.

The apparent effect of the T51E mutation on macroscopic (but not single-channel) rectification can be attributed to its effect on the voltage-dependent gating of the channel. At normal resting potentials ($V_m = -70$ mV) and a bath pH between 7.4 and 7.8, WT-ROMK2 exhibits a high open probability ($P_o = 0.8$) that increases slightly with membrane depolarization (solid line, Fig. 14). Although the P_o of T51E is similar to wild type at large negative potentials (see Figs. 9 and 10), it declines with depolarization, and at normal cell potentials it is only ~ 0.25 compared with 0.8 for wild type (dashed line, Fig. 14). This voltage dependence of the P_o (Fig. 14) can explain the apparent increased inward rectification of the T51E macroscopic currents (Fig. 13), since macroscopic conductance (G) is the product of single-channel conductance (g), number of channels (N), and P_o .

Single-channel properties of other substitutions at T51. The striking effect on voltage gating produced by replacing the threonine at position 51 with a negatively charged glutamate raised the possibility that other amino acid substitutions at this location might also affect channel gating and/or conductance. In this regard, Table 4 compares currents for wild type, T51E, T51H, and T51K, all obtained from one-channel, cell-

Fig. 6. Hydropathy analysis of the predicted amino acid sequence of ROMK2, based on the Kyte-Doolittle algorithm. *Abscissa*: amino acid sequence number. *Ordinate*: positive numbers denote increasing hydrophobicity. M1 and M2, putative membrane spanning region; P, pore region; and Q, area of mild hydrophobicity, explored in the present treatment.



attached patches on oocytes clamped at -100 mV relative to the pipette.

At -100 mV, T51E currents had lower amplitude and lower open probability than wild type, analogous to results obtained at -200 mV (Fig. 9). On the other hand, replacing the threonine at *position 51* with either a histidine (T51H) or a positively charged lysine (T51K) produced K currents that exhibited many of the features of WT-ROMK, such as clear channel openings and low open-channel noise. However, T51K and T51H currents also showed some important differences. First of all, T51H had a significantly higher single-channel conductance than WT-ROMK2, although its P_o was identical to that of WT-ROMK (Table 4). Second, T51K had a significantly lower P_o than either wild type or T51H, although its single-channel conductance was indistinguishable from wild type. Finally, depolarization from -100 mV to -50 mV reduced the P_o of T51K from 0.32 to 0.22. This type of voltage gating is opposite to that of WT-ROMK but similar to the voltage gating of T51E.

DISCUSSION

Modulation of macroscopic pH sensitivity. Patch-clamp studies on cut-open rat CCT initially established the regulation of secretory SK potassium channels by pH_i (15). This dependence on pH_i was also observed with ROMK channels expressed in oocytes and studied either at the single channel (10, 11) or the whole cell level (14). In the latter experiments, simultaneous measurement of macroscopic current and pH_i indicated a steep dependence of ROMK1 conductance on internal pH, involving a highly cooperative process with four H^+ binding sites and a pK_a of 6.8 (14). This is in contrast to the pH insensitivity of the strong inward rectifiers like IRK1 (4).

Recently, it was discovered that a single lysine (K61 on ROMK2, K80 on ROMK1), NH_2 terminal to the first hydrophobic segment (M1), is necessary for conferring pH_i sensitivity to ROMK (4). This result was confirmed in the present study, where replacing the lysine at

Q region

		44				51				59							
ROMK2	($K_{ir1.1b}$)	I	F	F	V	D	I	W	T	T	V	L	D	L	K	W	R
IRK1	($K_{ir2.1}$)	R	Y	L	A	D	I	F	T	T	C	V	D	I	R	W	R
mbIRK2	($K_{ir2.2}$)	R	Y	L	A	D	I	F	T	T	C	V	D	I	R	W	R
mbIRK3	($K_{ir2.3}$)	R	Y	M	A	D	I	F	T	T	C	V	D	T	R	W	R
GIRK1	($K_{ir3.1}$)	R	Y	L	S	D	L	F	T	T	L	V	D	L	K	W	R
mGIRK2	($K_{ir3.2}$)	R	Y	L	T	D	I	F	T	T	L	V	D	L	K	W	R
mGIRK3	($K_{ir3.3}$)	R	Y	L	T	D	L	F	T	T	L	V	D	L	Q	W	R
CIR	($K_{ir3.4}$)	R	Y	L	S	D	L	F	T	T	L	V	D	L	K	W	R
BIR10	($K_{ir4.1}$)	L	Y	L	K	D	L	W	T	T	F	I	D	M	Q	W	R
BIR9	($K_{ir5.1}$)	S	Y	M	V	D	I	F	T	T	L	V	D	T	K	W	R
uKATP-1	($K_{ir6.1}$)	R	F	L	Q	D	I	F	T	T	L	V	D	L	K	W	R
BIR	($K_{ir6.2}$)	R	F	L	Q	D	V	F	T	T	L	V	D	L	K	W	P

Fig. 7. Homologies within the "Q" regions of ROMK2 and the inward rectifier family. Boldface letters indicate residues identical to those of ROMK2. Boxed residues depict the locus of the T51E and T52E mutations. Numbers 44, 51, and 59 refer to amino acid locations along the ROMK2 sequence.

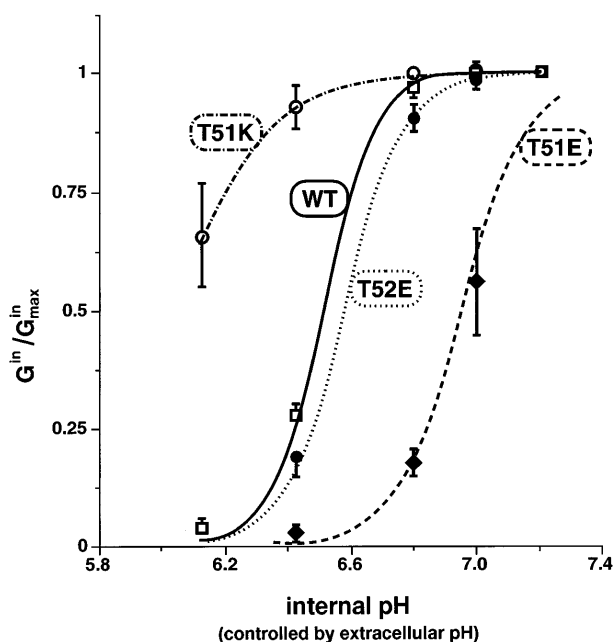


Fig. 8. pH dependence of WT-ROMK2 and T51 mutants. Ordinate: normalized inward conductance (at -80 mV) of oocytes expressing either WT-ROMK (WT, solid line, \square) or mutants T51E (dashed line, \blacklozenge) or T52E (dotted line, \bullet), or T52K (broken line, \circ). Conductances at different values of internal pH were normalized to the maximum inward conductance for that oocyte. Data were fit to a modified Hill equation (see text). Abscissa: pH_i controlled with extracellular potassium acetate solutions. Relationship between internal and external pH is given in Table 1.

position 61 with methionine (K61M) abolished the pH_i sensitivity of ROMK2 between 7.2 and 6.2. In addition to the critical residue K61, a second site (*position 51* on ROMK2; 70 on ROMK1) was also found to affect pH sensitivity of the channel. This might indicate a physical proximity between *positions 51* and *61* (on ROMK2) since changing the charge of *residue 51* shifted the apparent pK_a of the channel. Replacing the uncharged threonine by a negatively charged glutamate shifted the pK_a in an alkaline direction by 0.5 pH units, from 6.5 ± 0.01 (WT) to 7.0 ± 0.02 (T51E). Replacing threonine by a positively charged lysine shifted the pK_a in an acidic direction by 0.5 pH units, from 6.5 ± 0.01 (WT) to 6.0 ± 0.02 (T51K). However, the modulatory

Table 3. *Q* region mutants and whole cell pH sensitivity of ROMK clones

Clone	pK_a	n	No. of Oocytes
WT-ROMK2	$6.5 \pm .01$	$5.2 \pm .6$	8
T51E	$7.0 \pm .02^*$	4.1 ± 1	5
T52E	$6.6 \pm .01$	$4.5 \pm .2$	4
T51K	$6.0 \pm .02^*$	$2.8 \pm .3^*$	9
K61M	No fit		6
IRK1	No fit		5

pH dependence of the clones was fit to a modified Hill equation: $G/G_{\text{max}} = 1/[1 + ([\text{H}]/K)^n]$, where G is inward conductance; G_{max} is maximum inward conductance, $[\text{H}]$ is the internal hydrogen ion concentration, n is the Hill coefficient, and $\text{pK}_a = -\log_{10}K$. *Significantly different ($P < 0.05$) from wild-type ROMK2 (WT-ROMK2). "No fit" indicates no acceptable fit.

effects of *position 51* were not observed after removal of the critical lysine (K61); i.e., the double mutant T51E/K61M had the same insensitivity to pH as the single-point mutant (K61M, ROMK2). This suggests that the T51 locus modulates the pH sensitivity of the K61 site rather than functioning as an additional pH sensor.

Since the pK_a of lysine in free solution is 10.5, it was suggested that residue K61 confers pH sensitivity to the channel in the physiological range because the local chemical environment within the protein shifts the pK_a of lysine from 10.5 to ~ 6.5 , (4). How residue T51 modulates this pH sensitivity is not known. One hypothesis consistent with our results is that the mildly hydrophobic region at T51 is sufficiently close to the hydrophobic region of the first transmembrane segment (M1) to physically interact with K61, which is just NH_2 terminal to M1. The interaction could be electrostatic in nature, since positive and negative charges had opposite effects.

We believe that intracellular rather than extracellular pH modulates both the K61 and the T51 sites on ROMK2. We observed no obvious effect on ROMK macroscopic inward currents when bath pH was changed with membrane-impermeable KCl-HEPES solutions. A lack of effect of extracellular pH was also reported for ROMK1, where changes in bath pH via membrane-impermeable biphthalate had no effect on macroscopic inward currents (14).

Effect of pH on single-channel currents. The mechanism whereby decreases in cytoplasmic-side pH shut down channel activity is not well understood. In cell-attached patches, no ostensible change in channel kinetics occurs until ~ 10 – 15 min after reducing bath pH to 6.8. Presumably, this is the time required for the interior of the oocyte to reach a critical internal pH of ~ 6.4 . At this pH_i a very long closed state appears (Fig. 2B), which eventually becomes a complete closure. For reasons we do not understand, complete channel closure at low pH_i for more than 45 s seems to interfere with reactivation upon return to a pH 7.4 bath solution. Although channels may continue to remain closed for a variable time after return to normal bath pH, the time spent at low pH seems to be a critical factor for restoration of channel activity.

Recordings from excised patches support the hypothesis that lowering cytoplasmic-side pH initiates very long closures, followed by complete cessation of channel activity. In excised patches, the decline in channel activity is slower than the decline in bath pH. It is unclear whether this represents a delay in diffusion of new solution to the patch of membrane within the pipette or simply a delayed effect of pH on channel gating.

Upon return of the bath to pH 7.4, ROMK2 channel activity recovered with a latency that varied between about a minute for excised patches to more than 15 min for cell-attached recordings. The delay for the cell-attached experiments presumably represents the time required to elevate oocyte pH back to normal with the membrane-permeable acetate solutions. However, a

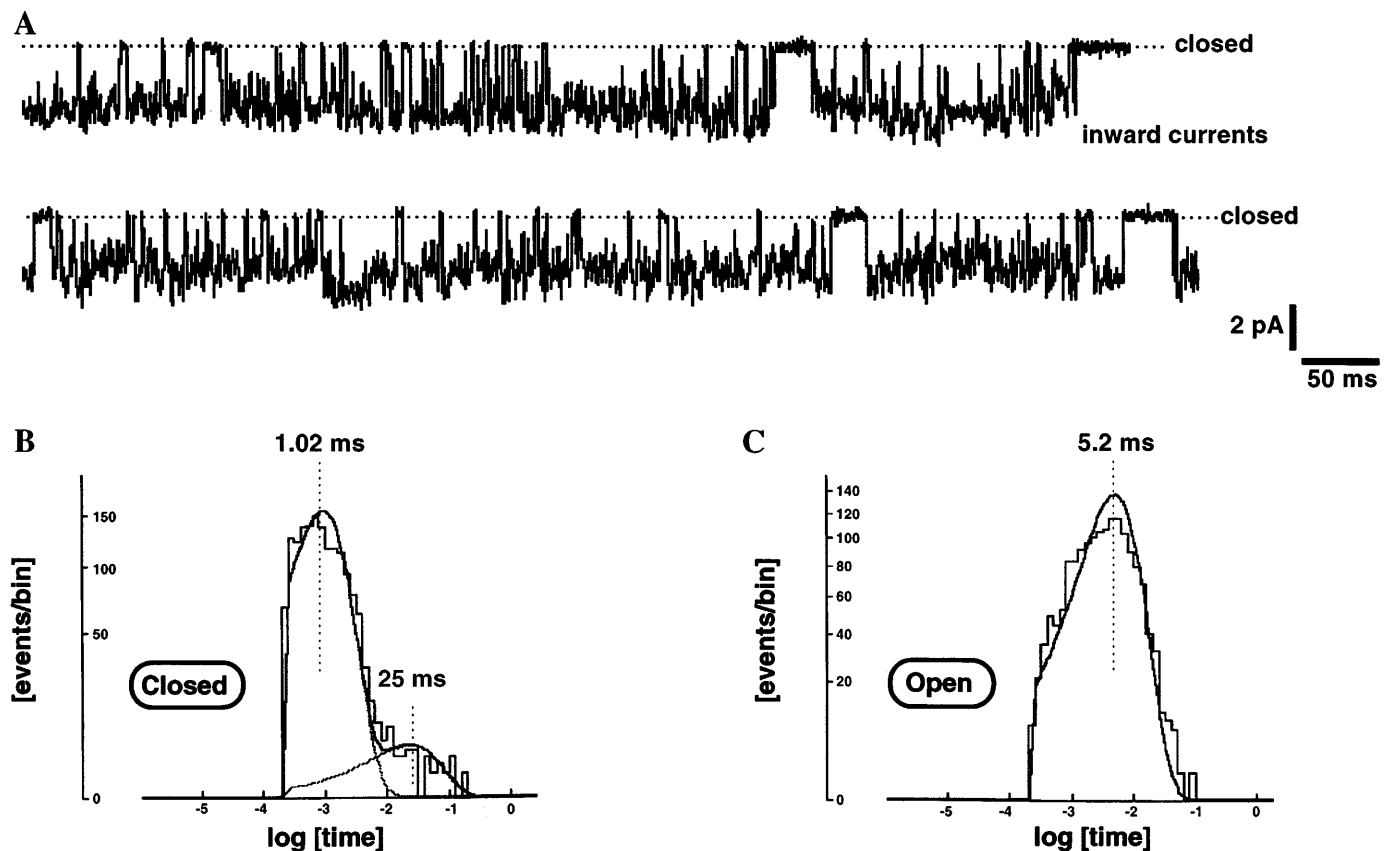


Fig. 9. K currents through a single T51E mutant ROMK2 channel, expressed in an oocyte, depolarized by 110 mM KCl (pH 7.4). *A*: cell-attached patch with oocyte potential of -200 mV relative to pipette. Downward deflections from the closed state (dotted line) denote inward currents. Inward single-channel conductance was 16 pS. *B*: closed time distribution for T51E, showing 2 discrete closed states with time constants of 1.0 and 25 ms. *C*: open time distribution for T51E currents, showing 1 open state with time constant of 5.2 ms. P_o (-200 mV) = 0.74.

delay in recovery of channel activity was also observed in excised patches, suggesting that the reversal of the inhibited state is slow compared with the actual change in pH.

Exposure of either excised or cell-attached patches for longer than 45 s prevented restoration of channel activity following a return to pH 7.4. The relatively narrow time window (45 s) beyond which it became impossible to reactivate individual single channels differs from the reversibility of WT-ROMK macroscopic conductance measured with the TEVC. In these latter experiments, inward conductance essentially returned to control values even after a 10-min exposure to a pH 6.8 bath. The reason for this difference in microscopic vs. macroscopic reversibility is not known.

Single-channel properties of the T51 mutants. The position of residue T51 (ROMK2) relative to the first transmembrane segment raised the possibility that this site might also interact with the K permeation path of the channel. In fact, the T51E mutation reduces apparent single-channel conductance by 50% and dramatically increases open-channel noise. These changes are consistent with an interaction between *residue 51* and the permeation path of the channel.

The persistence of T51E-specific open-channel noise at all voltages and over a range of pH_i from 6.1 to 7.4

seems to rule out cytoplasmic proton block as a cause of the increased noise level. However, it is still possible that the T51E mutation introduces a fast-flicker open state that is beyond the resolution of the data acquisition system. In this case, T51E could appear to have a much reduced conductance when records are filtered at 1 kHz.

Although the reason for the decrease in apparent conductance produced by T51E is not known, it is unlikely that it arises from a simple electrostatic effect, since replacing threonine with a positively charged basic lysine residue (T51K) did not produce an opposite effect on conductance. In fact, the single-channel conductance of T51K was indistinguishable from that of WT-ROMK2 (Table 4). Furthermore, when the T51 residue was replaced by histidine (which should be less protonated than lysine at pH = 7.4), single-channel conductance actually increased (Table 4).

We have no hypothesis for the dramatic effect of the glutamate substitution (T51E) on single-channel voltage gating (Fig. 14). Other amino acid substitutions at this position, T51H for example, leave P_o virtually unchanged from wild type (Table 4). A simple electrostatic mechanism seems an even less likely explanation for changes in voltage gating, since the T51K mutant (which introduces a positive charge at *position 51*) also

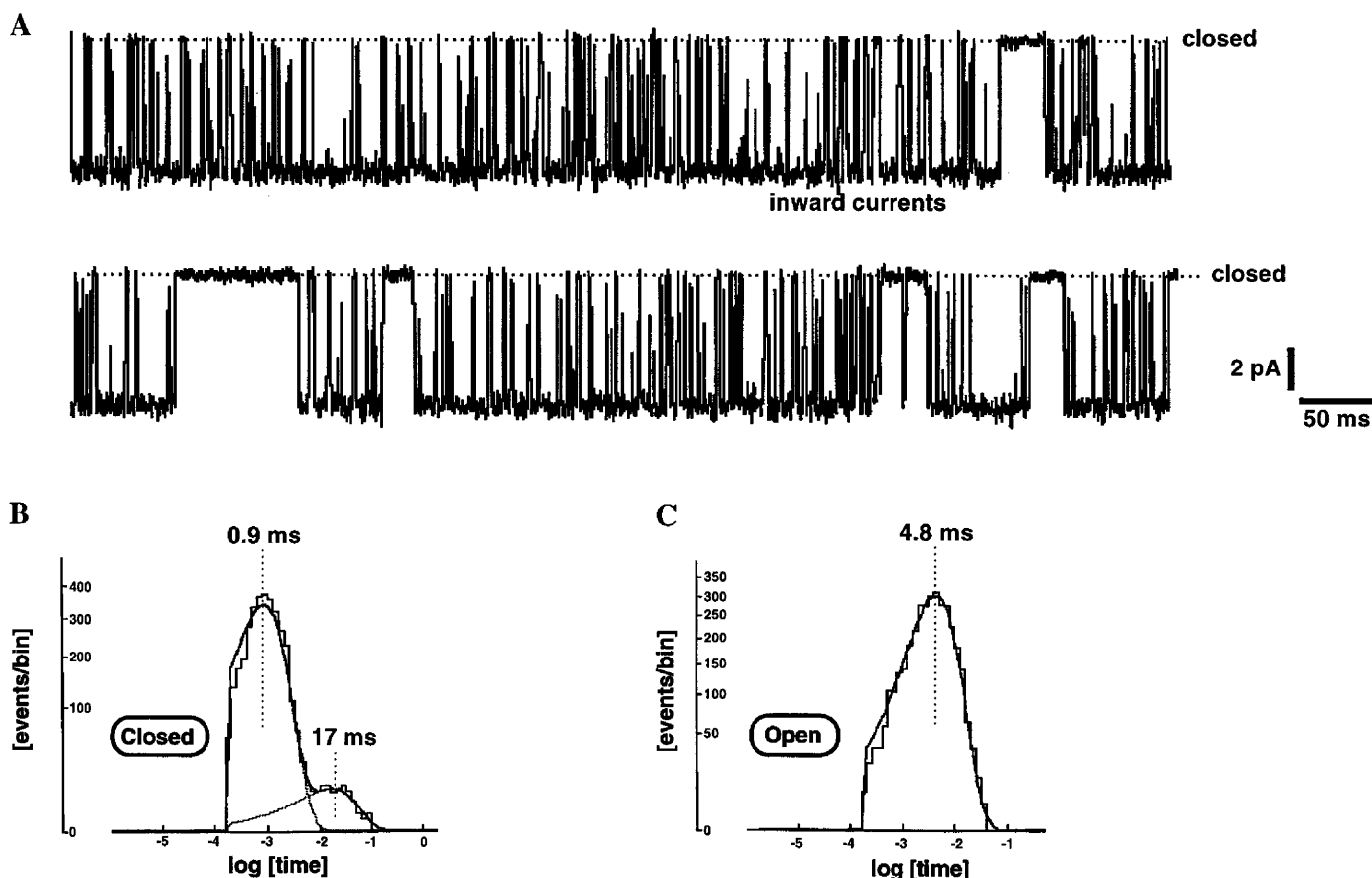


Fig. 10. K currents through a single WT-ROMK2 channel, expressed in an oocyte, depolarized by 110 mM KCl (pH 7.4). *A*: cell-attached patch with oocyte potential of -200 mV relative to pipette. Downward deflections from the closed state (dotted line) denote inward currents. Inward single-channel conductance was 33 pS. *B*: closed time distribution for WT-ROMK, showing 2 discrete closed states with time constants of 0.9 and 17 ms. *C*: open time distribution for WT-ROMK, showing 1 open state with time constant of 4.8 ms. P_o (-200 mV) = 0.72.

decreases the P_o of the channel, in a manner similar to that seen with (the negatively charged) T51E.

Comparison with previous studies. The present study confirms previous findings on the marked dependence of ROMK current on pH_i (4, 10, 11, 14, 15). We have also confirmed that the lysine residue (K61 on ROMK2, K80 on ROMK1), NH_2 terminal to the first putative transmembrane segment (M1), is primarily responsible for conferring a steep pH sensitivity to ROMK that is not seen with IRK1 (4).

The principal difference between our pH dependence of WT-ROMK and that reported by both Tsai et al. (14) and Fakler et al. (4) is the value of apparent pK_a for ROMK. In our studies the apparent pK_a for ROMK2 was 6.5 ± 0.01 . This is significantly lower than either the pK_a of 6.8 reported for ROMK1 by Tsai et al. (14) or the pK_a of 6.9 reported for ROMK2 by Fakler et al. (4). The precise reason for this disagreement is not known but probably arises from the larger discrepancy between intracellular and extracellular pH measured in our study, compared with previous reports (14).

In our experiments (Table 1), decreasing bath pH from 7.4 to 6.8 reduced oocyte pH from 6.8 to 6.4 compared with the decrease in pH_i of 7.2 to 6.7 reported by Tsai et al. (14). Furthermore, we were never able to

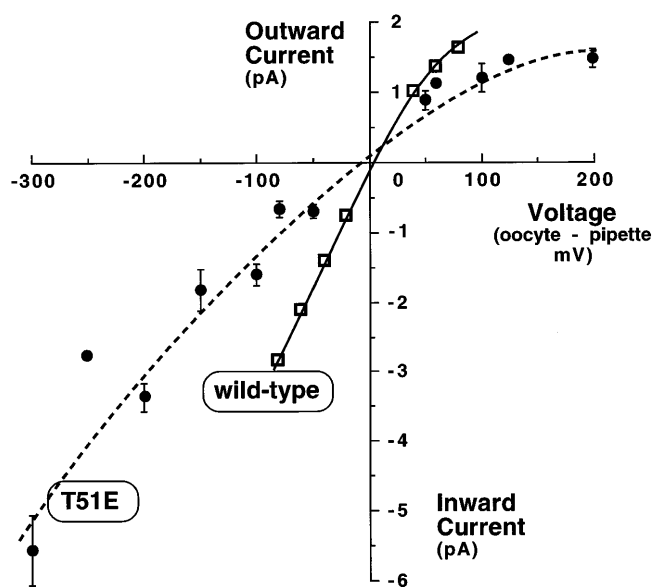


Fig. 11. Comparison of single-channel rectification between T51E and WT-ROMK2. Cell-attached patches on oocytes expressing either T51E mutant (dashed line, ●) or WT-ROMK (solid line, □). Pipette and bath solutions consisted of 110 mM KCl buffered to pH 7.4. Pipette also contained 1 mM $MgCl_2$. Mean inward single-channel conductance for T51E was 17 ± 1 pS ($n = 9$). Mean inward single-channel conductance for wild type was 34 ± 1 pS ($n = 8$).

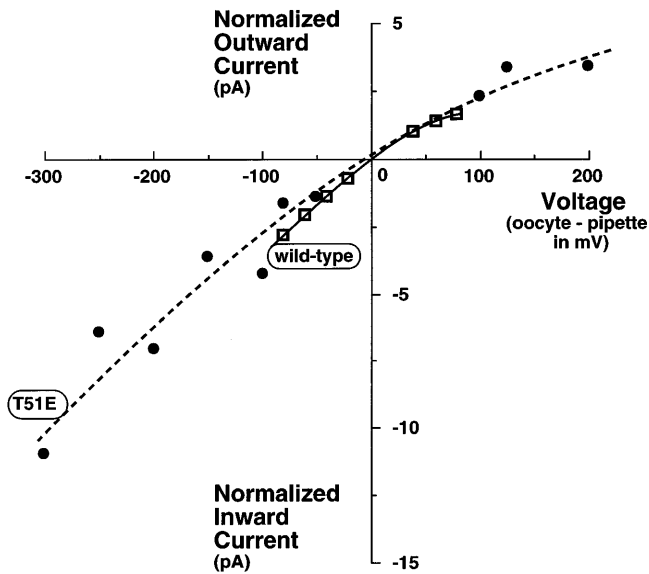


Fig. 12. Normalized single-channel current-voltage (I - V) relations for T51E and wild type. Cell-attached patches on oocytes expressing either T51E mutant (dashed line, ●) or WT-ROMK (solid line, □). Pipette and bath solutions consisted of 110 mM KCl buffered to pH 7.4. Pipette also contained 1 mM MgCl₂. Data are from Fig. 11, where T51E currents have been scaled by the WT/T51E ratio of inward conductances at -80 mV.

alkalinize oocytes above their resting pH (about 7.2) using acetate-buffered solutions, so that it was only possible (with this technique) to study oocytes with an internal pH less than or equal to 7.2.

The disparity in pK_a values is of interest, since it determines whether ROMK2 would be regulated by normal variations in pH_i . The results of our study

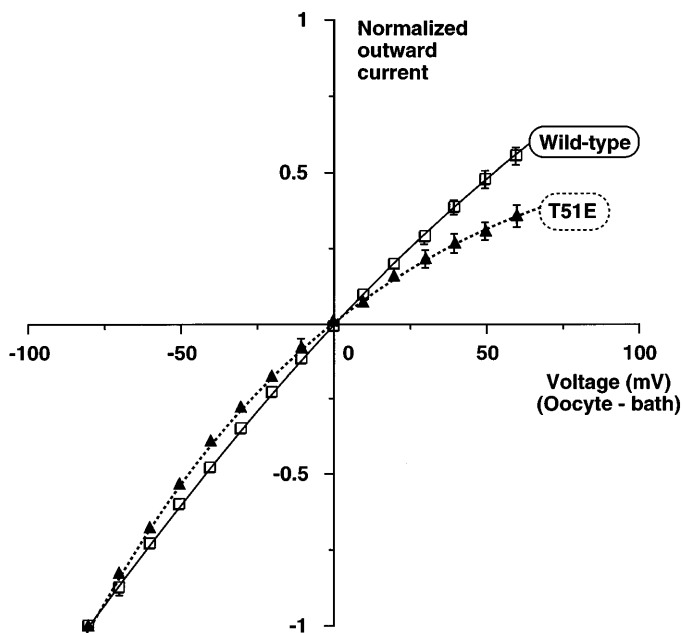


Fig. 13. T51E enhances macroscopic inward rectification. Comparison of the 2-electrode I - V relations for the mutant T51E and WT-ROMK2. All currents were normalized to maximum inward current at -80 mV. Bathing solution contained 110 mM KCl (pH = 7.4), and the average oocyte resting potential was zero mV.

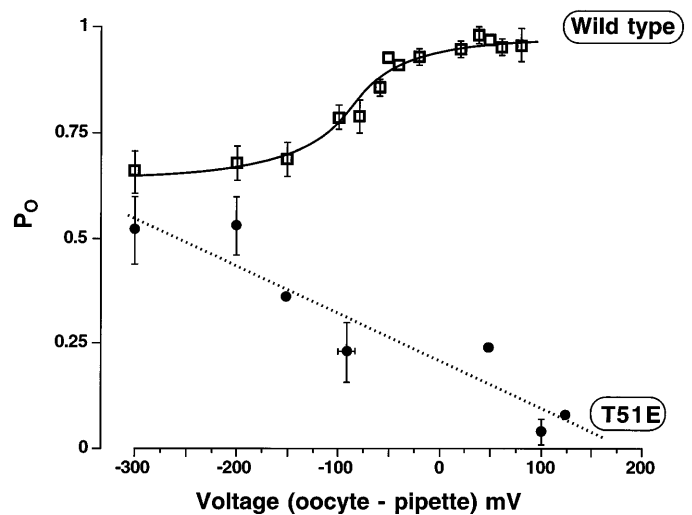


Fig. 14. Voltage dependence of open probability for T51E and wild type. Cell-attached patches on oocytes expressing either T51E mutant (dashed line, ●) or WT-ROMK2 (solid line, □). Pipette and bath solutions consisted of 110 mM KCl buffered to pH 7.4. Pipette also contained 1 mM MgCl₂.

suggest that ROMK2 is probably not significantly regulated by pH under physiological conditions, since it is unlikely that renal cells would normally possess a pH_i below 6.7. Hence, only those cells with impaired acid extrusion processes would have a sufficiently low pH_i to shut down the secretory ROMK channel. This might serve as a protective mechanism to conserve intracellular K during energetically restricted conditions in which pH_i regulation was compromised.

Summary. The present study confirms that a lysine residue (K61 on ROMK2; K80 on ROMK1) is the primary locus of the pH sensitivity present in ROMK but not in IRK1. However, a specific residue in a mildly hydrophobic region of ROMK (T51 on ROMK2; T70 on ROMK1) appears to modulate this pH sensitivity. Replacing the neutral threonine at this site with a negatively charged residue shifts the apparent pK_a in an alkaline direction, whereas substitution with a positively charged residue shifts the pK_a in an acid direction. Substitutions at *position 51* also significantly affected single-channel conductance and voltage-dependent gating of the channel. In contrast, mutations in a neighboring residue T52 (T71 on ROMK1) had no effect on pK_a or single-channel conductance. This is consistent with a model in which T51 is sufficiently close to the primary pH site (K61) to alter ROMK pH sensitiv-

Table 4. *Single-channel conductance and P_o of WT-ROMK and T51 mutants*

Clone	n	g_{inward} , pS	P_o (-100 mV)
WT-ROMK2	8	34.0 ± 1.0	0.76 ± 0.04
T51E	9	17.4 ± 1.0*	0.22 ± 0.07*
T51K	4	36.4 ± 2.0	0.32 ± 0.02*
T51H	2	40.5 ± 3.0*	0.73 ± 0.08

Values are means ± SE; n = numbers of oocytes. Cell-attached patches on oocytes expressing the indicated clone. Pipette and bath solutions contained 110 mM KCl. Bath pH = 7.4. *Significantly different ($P < 0.05$) from WT-ROMK2.

ity while also exerting effects on the permeation pathway in the pore region.

We gratefully acknowledge the expert assistance of Dr. Gordon Cooper, Dr. Michael Romero, and Dr. Walter Boron with the intracellular measurements of oocyte pH, which were all performed in the Dept. of Cellular and Molecular Physiology at Yale University School of Medicine.

This work was supported by a grant-in-aid from the American Heart Association/New York City Affiliate (to L. G. Palmer) and by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-46950 (to H. Sackin).

Address for reprint requests: H. Sackin, Yale University School of Medicine, 2073 LMP, 333 Cedar St., New Haven, CT 06510.

Received 20 December 1996; accepted in final form 27 May 1997.

REFERENCES

1. **Boim, M. A., K. Ho, M. E. Shuck, M. J. Bienkowski, J. H. Block, J. L. Slightom, Y. Yang, B. M. Brenner, and S. C. Hebert.** ROMK inwardly rectifying ATP-sensitive K⁺ channel. II. Cloning and distribution of alternative forms. *Am. J. Physiol.* 268 (*Renal Fluid Electrolyte Physiol.* 37): F1132–F1140, 1995.
2. **Chepilko, S., H. Zhou, H. Sackin, and L. G. Palmer.** Permeation and gating properties of a cloned renal K⁺ channel. *Am. J. Physiol.* 268 (*Cell Physiol.* 37): C389–C401, 1995.
3. **Choe, H., and H. Sackin.** Improved preparation of *Xenopus* oocytes for patch-clamp recording. *Pflügers Arch.* 433: 648–652, 1997.
4. **Fakler, B., J. Schultz, J. Yang, U. Schulte, U. Brändle, H. P. Zenner, L. Y. Jan, and J. P. Ruppertsberg.** Identification of a titratable lysine residue that determines sensitivity of kidney potassium channels (ROMK) to intracellular pH. *EMBO J.* 15: 4093–4099, 1996.
5. **Frindt, G., and L. G. Palmer.** Low-conductance K channels in apical membrane of rat cortical collecting tubule. *Am. J. Physiol.* 256 (*Renal Fluid Electrolyte Physiol.* 25): F143–F151, 1989.
6. **Frindt, G., R. B. Silver, E. E. Windhager, and L. G. Palmer.** Feedback inhibition of Na channels in rat CCT. II. Effects of inhibition of Na entry. *Am. J. Physiol.* 264 (*Renal Fluid Electrolyte Physiol.* 33): F565–F574, 1993.
7. **Glowatzki, E., G. Fakler, U. Brändle, U. Rexhausen, H. P. Zenner, J. Ruppertsberg, and B. Fakler.** Subunit-dependent assembly of inward-rectifier K channels. *Proc. R. Soc. Lond. B Biol. Sci.* 261: 251–261, 1995.
8. **Ho, K. H., C. G. Nichols, W. J. Lederer, J. Lytton, P. M. Vassilev, M. V. Kanazirska, and S. C. Hebert.** Cloning and expression of an inwardly rectifying ATP-regulated potassium channel. *Nature* 362: 31–37, 1993.
9. **Ho, S. N., H. D. Hunt, R. M. Horton, J. K. Pullen, and L. R. Pease.** Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene* 77: 51–59, 1989.
10. **McNicholas, C. M., W. B. Guggino, E. M. Schwiebert, S. C. Hebert, G. Giebisch, and M. E. Egan.** Sensitivity of a renal K channel (ROMK2) to the inhibitory sulfonylurea compound glibenclamide is enhanced by coexpression with the ATP-binding cassette transporter cystic fibrosis transmembrane regulator. *Proc. Natl. Acad. Sci. USA* 93: 8083–8088, 1996.
11. **McNicholas, C. M., G. G. MacGregor, M. W. Nason, Y. Yang, G. Giebisch, and S. C. Hebert.** Mechanism of pH dependent modulation of ROMK K channel activity (Abstract). *J. Am. Soc. Nephrol.* 7: 1286, 1996.
12. **Palmer, L. G., and G. Frindt.** Conductance and gating of epithelial Na channels from rat cortical collecting tubules. Effects of luminal Na and Li. *J. Gen. Physiol.* 92: 121–138, 1988.
13. **Stanton, B. A., and G. Giebisch.** Potassium transport by the renal distal tubule: effects of potassium loading. *Am. J. Physiol.* 243 (*Renal Fluid Electrolyte Physiol.* 12): F487–F493, 1982.
14. **Tsai, T. D., M. E. Shuck, D. P. Thompson, M. J. Bienkowski, and K. S. Lee.** Intracellular H⁺ inhibits a cloned rat kidney outer medulla K⁺ channel expressed in *Xenopus* oocytes. *Am. J. Physiol.* 268 (*Cell Physiol.* 37): C1173–C1178, 1995.
15. **Wang, W., A. Schwab, and G. Giebisch.** Regulation of small conductance K channel in apical membrane of rat cortical collecting tubule. *Am. J. Physiol.* 259 (*Renal Fluid Electrolyte Physiol.* 28): F494–F502, 1990.
16. **Wright, F. S., and G. Giebisch.** Regulation of potassium secretion. In: *The Kidney: Physiology and Pathophysiology* (2nd ed.), edited by D. W. Seldin and G. Giebisch. New York: Raven, 1992, p. 2209–2247.
17. **Yang, J., Y. N. Jan, and L. Y. Jan.** Determination of the subunit stoichiometry of an inwardly rectifying potassium channel. *Neuron* 15: 1441–1447, 1995.
18. **Zhou, H., S. S. Tate, and L. G. Palmer.** Primary structure and functional properties of an epithelial K channel. *Am. J. Physiol.* 266 (*Cell Physiol.* 35): C809–C824, 1994.