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External pH regulates the slowly activating potassium current I_{sK} expressed in *Xenopus* oocytes

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A slowly activating, delayed rectifier potassium current, I_{sK}, was expressed in *Xenopus laevis* oocytes by injection of cRNA transcribed from a rat kidney cDNA clone. External acidification reversibly decreased the current amplitude. The effects were concentration dependent on protons with K_d at pH≈5.5 and a Hill coefficient of 1.0. External acidification reduced the maximal conductance (G_{max}) without affecting the activation kinetics; this effect was not dependent on membrane voltages. These data suggest that H^+ ions bind to the channel with a one-to-one stoichiometry, and this binding site may be located outside of the membrane electric field.

I_{sK} channel; *Xenopus* oocyte; External pH; Single binding site

1. INTRODUCTION

Although raising the concentration of external H^+ ions has been shown to suppress delayed rectifier potassium currents in several cell types [7–9,13–15], the precise mechanism of the block has not been clarified yet. A novel type of the potassium channel from kidney, I_{sK}, which was originally cloned by functional expression, revealed a very slowly activating, voltage-dependent delayed rectifier-type K^+ current when the cRNA transcribed from the cDNA clone of this channel was injected into *Xenopus* oocytes [1]. This K^+ channel was first isolated from a rat kidney mainly localized in epithelial cells [1]. Thereafter it has also been isolated from a rat uterus, neonatal rat heart [11] and human genomic DNA [12]. The cloned nucleotide sequence of the channel encodes a small protein that consists of 130 amino acids with a single putative transmembrane domain different from the structure of the known voltage-dependent ion channel proteins. In this paper, we showed that the I_{sK} channel was modulated by decrease in the external pH similar to the known delayed rectifier K^+ channels defined by the electrophysiological experiments. Therefore we examined the mechanism of block by external protons on the expressed channel.

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2. MATERIALS AND METHODS

The sequence encoding the potassium channel was amplified by polymerase chain reaction from the rat kidney cDNA library using primers based on the sequence of Takumi et al. [1]. The amplified fragment was subcloned into pSPORT-1 (BRL) and sequenced. Capped mRNA was synthesized in vitro using T7 RNA polymerase and injected into *Xenopus laevis* oocytes by using a 10-μl Drummond micropipetter modified for microinjection (Drummond Scientific Co., Broomhall, PA). After the injection of 10–15 ng of RNA, the oocytes were incubated for 2–4 days at 20°C in modified Barth's solution before electrophysiological experiments.

Membrane currents were recorded by two electrode voltage clamp using a Dagan 8500 amplifier (Dagan Corp., Minneapolis, MN) at a room temperature of 20–22°C. The standard external solution (ND96) contained (mM): 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$ and 5 HEPES (pH 7.6). When we used the solution with different pH, the following buffers were used: HEPES (pK_a 7.5) for pH 7.6 and 6.6; MES (2-[*N*-morpholino]ethanesulfonic acid, pK_a 6.1) for pH 5.6; propionic acid (pK_a 4.9) for pH 4.6 and 3.6, and titrated to the final pH with HCl or NaOH. Shielded electrodes filled with 3 M KCl had resistances of 1–2 MΩ, when measured in ND96. At the start of each experiment, the resting membrane potential of oocyte was measured and the preparations showing the potential greater than −55 mV were chosen for further experiments (average: −63.3 ± 1.7 mV, $n = 7$). Time-dependent, outward potassium currents were evoked by applying depolarizing pulses for 8–10 s from a holding potential of −80 mV. To block the endogenous Ca^{2+}-dependent chloride current, 300 μM of SITS (4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid disodium salt, Sigma Chemical Co., St. Louis, MO) was added to the bath solution in all experiments. Several other currents are present in the endogenous *Xenopus* oocytes membrane [18]. In order to minimize the influence of the endogenous membrane currents, we subtracted the averaged current obtained in 7 H_2O-injected oocytes from the current traces of mRNA-injected oocytes at each voltage and pH. Since the current does not reach a steady-state value even with long depolarizing pulses of 8–10 s, both the rate of activation and the maximal current were estimated by fitting a single exponential function to the observed currents. Data were collected and analyzed using pClamp software (Axon Instruments Inc., Burlingame, CA). All the values were expressed as mean ± S.E.M. Statistical analysis was done using the

paired or unpaired t test where appropriate, and $P < 0.05$ was considered significant.

3. RESULTS

In the voltage-clamp experiment using oocytes that had been injected with the mRNA of the I_{sK} channel, time-dependent and slowly activating outward currents were elicited by depolarizing pulses from a holding potential of −80 mV to test voltages between −60 and +60 mV in 20-mV steps for 8 s at a 30-s interval (Fig. 1A). The reversal potentials were estimated with the tail currents upon repolarization to various voltages from the 10 s test depolarization to +20 mV (not shown). They were around −90 and −40 mV with the external K^+ concentrations of 2 and 21 mM, respectively, which were close to the expected value of the K^+ equilibrium potential from the Nernst equation.

When the external solution was changed to acidic solution, current amplitudes were decreased promptly (in a few minutes) as shown in Fig. 1B and the changes were reversible when external pH was normalized. Although effects of external proton was concentration-dependent as shown in Fig. 2, in which the relative current amplitude was plotted against pH value, no voltage dependence of block was observed within the voltage range between 0 and +60 mV. The data were fitted by a least-squares analysis according to a Hill equation in the form of:

$$I/I_{max} = 1/[1 + (10^{-pH}/K_d)^n]$$

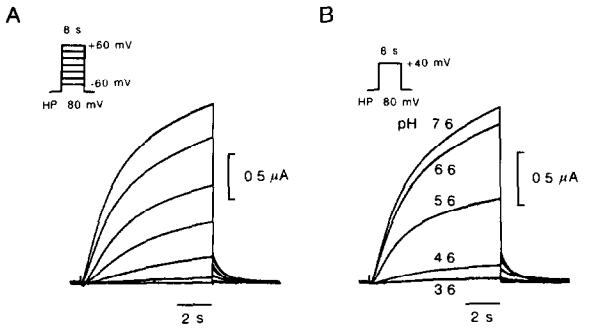

Fig. 1. (A) Membrane currents evoked by depolarizing steps in an oocyte injected with RNA encoding the I_{sK} channel. Currents were elicited by step pulse protocols shown in the inset on the upper left corner; 8-s-long depolarizing pulses were applied from a holding potential of −80 mV to test voltages between −60 and +60 mV in 20 mV increments. The external solution was the standard ND96 (pH 7.6). (B) Regulation of the I_{sK} channel by external acidification. Traces of the I_{sK} recorded in the external solution with pH of 7.6, 6.6, 5.6, 4.6, and 3.6 are superimposed and displayed in the order of the uppermost trace with pH 7.6 to the lowest trace with pH 3.6. All the currents were elicited by a 8 s depolarizing pulse +40 mV as shown in the inset on the upper left corner. A and B were obtained from the same oocyte.

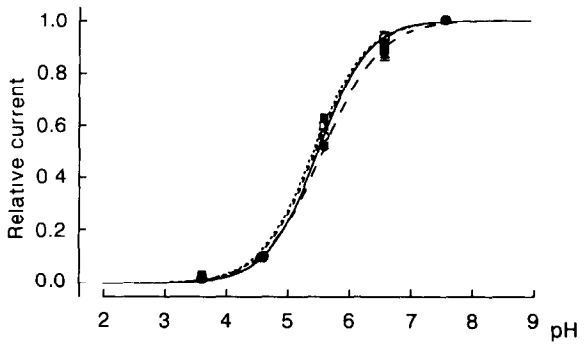

Fig. 2. The concentration-dependent block of I_{sK} by protons at different voltages. The relative current amplitude expressed as a fraction of the value at pH 7.6 was plotted against the external pH value. Lines were obtained by fitting of Hill equation to the data with K_d values of pH 5.42, 5.45, 5.58, and 5.50 at the membrane voltage of 0 (■), +20 (□), +40 (●), and +60 mV (○), respectively. The K_d values and lines were not different and superimposable. Voltage protocol was the same as the one shown in Fig. 1A. Values and bars indicate the mean ± S.E.M. of 7 experiments, if the S.E.M. values were larger than the symbol.

where K_d is the dissociation constant, n is the Hill coefficient, and I_{max} is the current value when pH was 7.6. The half-maximal inhibitions were achieved at pH values of 5.42, 5.45, 5.58, and 5.50 at the membrane voltage of 0, +20, +40, and +60 mV, respectively. The n value (Hill coefficient) was close to a unity at each voltage (1.003, 0.998, 0.923, and 1.052 at 0, +20, +40, and +60 mV, respectively).

The conductance of this channel was decreased by about 40% when the external pH was reduced from 7.6 to 5.6 as shown in Fig. 3A. Since the decrease of the conductance was about the same degree at all the potentials examined, the activation-curve was barely shifted along the voltage axis (Fig. 3B). In both Fig. 3A and B, lines are drawn by the fits to Boltzmann equation:

$$f_\infty = G_{max} - G_{max}/\{1 + \exp[(V_m - V_h)/s]\} \text{ in Fig. 3A;}$$

$$f_\infty = 1 - 1/\{1 + \exp[(V_m - V_h)/s]\} \text{ in Fig. 3B}$$

where V_h is the membrane voltage at which conductances become half-maximal, and s is a slope factor. The averaged value of V_h was -6.03 ± 1.68 mV and -8.50 ± 2.21 mV, and s was 15.86 ± 0.76 and 16.75 ± 0.53 at pH 7.6 and 5.6, respectively. There was no significant difference between them.

Fig. 4 shows the relationship between the membrane potential and the time constant for activation at three external pH conditions. As previously described [2], activation time course was well fitted to a single exponential function and the time constants were decreased as the test potentials were depolarized. A decrease in external pH showed little change in a time constant at each membrane voltage.

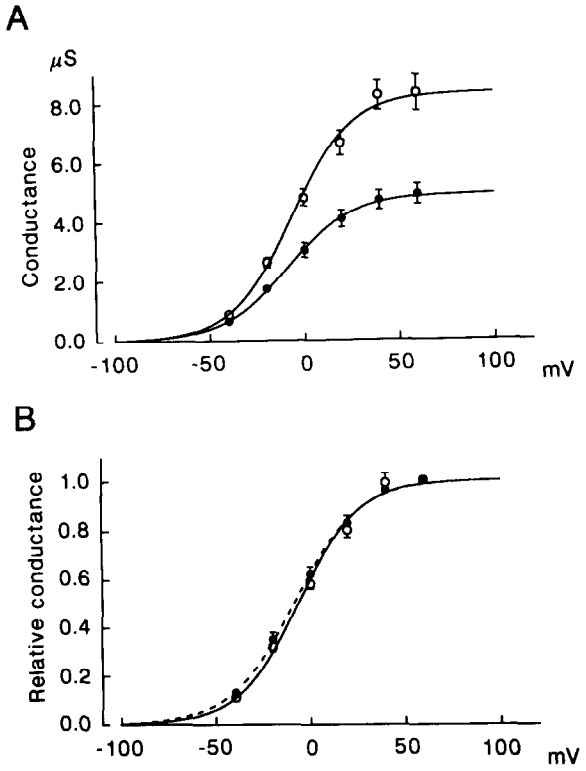

Fig. 3. Changes in conductances of the I_{sK} channel with external acidification. In panel A, the maximal conductance (G_{max}) was plotted against membrane voltages. When the external pH was reduced from 7.6 (○) to 5.6 (●), G_{max} was reduced by about 40% at each membrane potential. In panel B, relative conductance normalized by the value at +60 mV was plotted against membrane potentials. Activation curves did not shift along the voltage axis with lowering external pH. In both panel A and B, lines are the fits of the Boltzmann equation to the data with V_h values of −6.0 and −8.5 mV and s values of 15.9 and 16.8 with pH 7.6 (○) and 5.6 (●), respectively. Values and bars indicate the mean ± S.E.M. of 5 experiments.

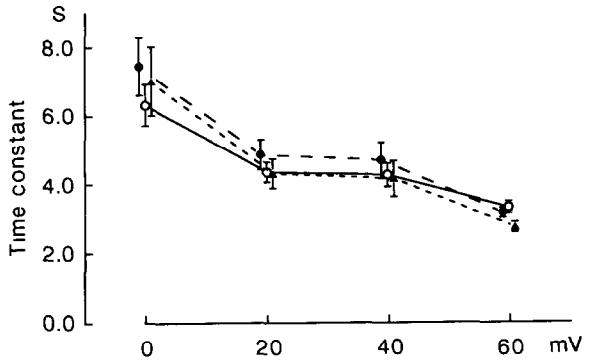

Fig. 4. Relationship between the membrane potential and the time constant for activation at three external pH conditions: 7.6 (○), 6.6 (●), and 5.6 (▲). Time constants did not change with external acidification. Values and bars indicate the mean ± S.E.M. of 7 experiments.

4. DISCUSSION

This study demonstrated that the slowly activating potassium current (I_{sK}) expressed in *Xenopus* oocytes from a rat kidney cDNA clone was affected by decrease in pH value of the extracellular solution. The main action of the increased external proton was to reduce the maximal potassium conductance (G_{max}) without affecting the activation kinetics of the channel which was estimated by the voltage-dependence of activation and the time constant of activation.

An increase in the external H^+ concentrations has been shown to exert at least two major effects on delayed rectifier K^+ currents in squid giant axon and frog node of Ranvier [7–9,13,19]. One effect is to shift the voltage dependence of channel activation in a depolarizing direction. This effect is thought to reflect the binding of H^+ ions to diffuse or aggregate negatively charged moieties near the outer mouth of the channels, altering the voltage field sensed by the channel gating mechanism. The other effect is to reduce the conductance of open channels in a manner suggesting the titration of one of acidic groups within the pore [8,16].

We observed that the activation kinetics did not change by reduction of external pH, which might suggest that changes in the surface charges do not affect the activation kinetics of this channel. Therefore, the effect on surface charges seems not to be involved in the mechanism of the suppression of the I_{sK} channel by protons. On the other hand, the maximal conductances were markedly reduced by external H^+ ions as shown in Fig. 3A. The decreased conductance may suggest the binding of H^+ ions to the channel pore [8,16]. However, the reduction in current conductances showed no voltage-dependency, suggesting that the binding site of H^+ ions was located outside of the membrane electric field. Furthermore, the Hill coefficient close to the unity suggests that protons bind to the I_{sK} channel with a one-to-one stoichiometry.

Although the hydropathy analysis indicated a single hydrophobic segment consisting of 23 uncharged amino acid residues in the middle portion of the protein [1], the real structure of this channel is still unknown; especially it is controversial whether the N-terminal locates intracellular or extracellular side. There are three acidic residues just proximal to the hydrophobic domain. Assuming that N-terminal is extracellular, these acidic residues locate outside of the membrane electric field and are good candidates for the binding site of H^+ ions. If this assumption is correct, it would be an excellent clue to study the structure–function relationship of the I_{sK} channel.

The I_{sK} channel protein was shown to be restrictedly localized in the apical membrane portion of epithelial cells in the renal proximal tubule, the submandibular duct and the uterine endometrium [6]. Although the function of I_{sK} channel has not been fully established yet, its role is considered at present as follows. The Na^+,K^+-ATPase pump located in the basolateral mem-

brane of epithelial cells generates a lower intracellular Na^+ concentration, and resultant Na^+ gradient across the apical membrane causes the entry of Na^+ ions into the cell from the lumen through the Na^+/sugar or amino acid cotransport system. This Na^+ entry depolarizes cells about 10–15 mV and stimulates the K^+ channel activity of the I_{sK} protein, and results in permeations of K^+ ions from the epithelial cells to the lumen [6,17]. The results of this work may suggest the accumulation of K^+ ions inside the epithelial cells at the time of acidification of luminal fluid although the functional significance of this phenomenon in vivo is not known yet.

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