Gating of I_{sK} Channels Expressed in *Xenopus* Oocytes

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ABSTRACT The channel underlying the slow component of the voltage-dependent delayed outward rectifier K^+ current, I_{Ks}, in heart is composed of the minK and K_vLQT1 proteins. Expression of the minK protein in *Xenopus* oocytes results in I_{Ks}-like currents, I_{sK}, due to coassembly with the endogenous XK_vLQT1. The kinetics and voltage-dependent characteristics of I_{sK} suggest a distinct mechanism for voltage-dependent gating. Currents recorded at 40 mV from holding potentials between −60 and −120 mV showed an unusual "cross-over," with the currents obtained from more depolarized holding potentials activating more slowly and deviating from the Cole-Moore prediction. Analysis of the current traces revealed two components with fast and slow kinetics that were not affected by the holding potential. Rather, the relative contribution of the fast component decreased with depolarized holding potentials. Deactivation and reactivation, after a short period of repolarization (100 ms), was markedly faster than the fast component of activation. These gating properties suggest a physiological mechanism by which cardiac I_{Ks} may suppress premature action potentials.

INTRODUCTION

The probability of opening for voltage-dependent ion channels is determined by the transmembrane potential. Channel activation results from a series of conformational transitions in the channel protein, particularly the movement of charged residues within the membrane electric field, which is manifested as gating currents (Armstrong and Bezanilla, 1973). For many voltage-gated channels, measurements of gating and ionic currents have provided insight into the different closed and open conformational states (Bezanilla et al., 1994; Stefani et al., 1994; Zagotta et al., 1994).

Expression of the minK protein in *Xenopus* oocytes results in voltage-dependent potassium currents, which activate much more slowly than other voltage-dependent channels, having time constants on the order of seconds. Recently it has been shown that the minK protein coassembles with K_vLQT1 (Sanguinetti et al., 1996; Barhanin et al., 1996), and currents recorded in *Xenopus* oocytes after injection of minK mRNA, I_{sK}, result from the coassembly of the minK protein with the endogenous XK_vLQT1 (Sanguinetti et al., 1996).

The remarkable structural and functional features of I_{sK} suggest a distinct mechanism for its voltage-dependent gating. In the experiments described here, we used ionic current measurements to examine different states that I_{sK} channels undergo during gating. The main findings of this study are that activation kinetics of I_{sK} channels depend on the holding potential and that reactivation is much faster than activation. These properties suggest a potential physiological role for native cardiac I_{Ks} under conditions that mimic fast reactivation, such as during rapid pacing or an early extrasystole in the heart. Our findings also show that activation cannot be explained by a sequential gating scheme that involves identical and independent states.

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MATERIALS AND METHODS

Xenopus laevis care and handling were as previously described (Christie et al., 1990). Briefly, ovaries were surgically removed, and oocytes were dissected apart in modified Barth's solution and defolliculated by digestion in calcium-free solution containing collagenase A. Oocytes were injected with RNA from a pressure injector and incubated at 18°C with rotary agitation in ND96 (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM HEPES, pH 7.4). Macroscopic currents were measured using a two-electrode voltage clamp with a CA-1 amplifier (Dagan Corp., Minneapolis, MN) interfaced to an LSI 11/73 computer (Digital Equipment Corp., Marlboro, MA). During recording, oocytes were continuously superfused with ND-96 at room temperature. To minimize variability in the kinetics of channel activation due to subunit density (Cui et al., 1994), we injected a fixed amount of minK cRNA (2 ng/oocyte), and only oocytes yielding current amplitude between 2 and 3 μA during 30-s steps to 40 mV, 2–5 days after injection, were used in this study.

Data analysis

The variability of values from experiments with multiple data points is presented as a mean ± SD.

RESULTS

Expression of the minK protein in *Xenopus* oocytes induced voltage-dependent potassium currents, which activated with a characteristic slow time course. Fig. 1 *A* shows two-electrode voltage-clamp recordings of a representative current family evoked by 30-s depolarizing commands, from a holding potential of −80 mV to test potentials from −60 to 40 mV. After depolarization to potentials more positive than −20 mV, the currents showed a sigmoidal delay in activation. The rising phase of activation after the sigmoidal delay was well fit with a double exponential, and the time constants for the two processes were plotted as a function of the test potential (Fig. 1 *B*). Both processes were voltage de-

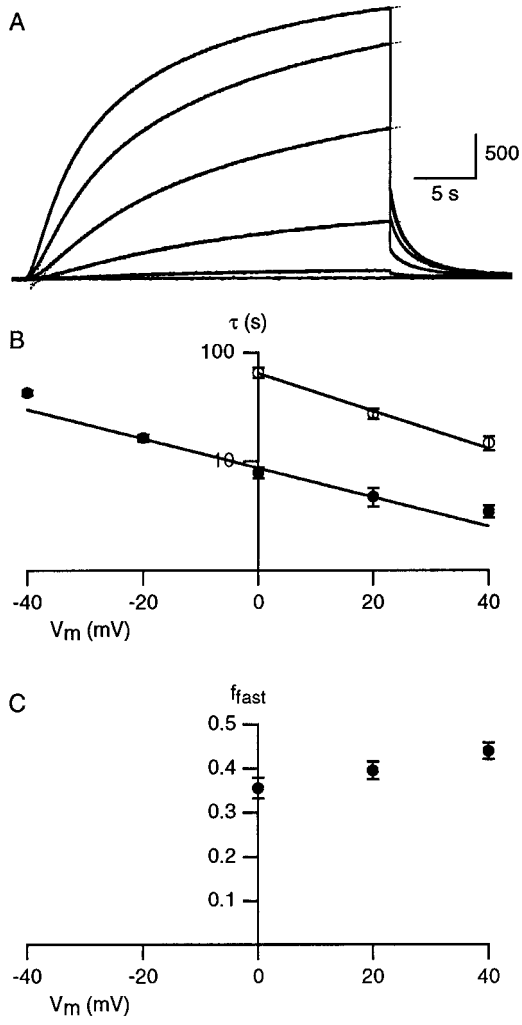

FIGURE 1 Activation kinetics of I_{sK}. (*A*) From a holding potential of −80 mV, currents were elicited by 30-s depolarizing commands to potentials from −60 to 40 mV in 20-mV increments, followed by repolarization to a tail potential of −60 mV. (*B*) Time constants for activation were derived from fits of a double exponential (for voltage pulses > -20 mV) or a single exponential (for voltage pulses ≤ -20 mV) to the rising phase of the current traces in *A* (·····). The mean time constants of the fast (●) and slow (○) component are plotted on a log scale as a function of the test potential. Data were fit with a single exponential yielding $66.7e^{-V/25.2}$ and $8.8e^{-V/32.3}$ for τ_{slow} and τ_{fast}, respectively. Bars represent the standard deviation from five experiments. (*C*) Relative amplitude of the fast component, f_{fast}, plotted against a test potential. f_{fast} is defined as $a_{fast}/(a_{fast} + a_{slow})$, where a_{fast} and a_{slow} are the amplitudes of the fast and slow component, respectively. Bars represent the standard deviation from five experiments.

pendent, and the relative contribution of the fast component increased with more depolarized voltages (Fig. 1 *C*). The time course of the tail currents after repolarization to −60 mV suggests that deactivation may be faster than activation (Fig. 1 *A*). Therefore, deactivation kinetics were measured by using a protocol in which the membrane potential was stepped to 40 mV for 20 s, followed by repolarizing test commands to potentials between −20 and −140 mV. For potentials between −20 and −80 mV, the tail currents were well fit by a double exponential, whereas for potentials more negative than −80 mV, the tail currents required only a single exponential (Fig. 2 *A*). The time constants were plotted as a function of the tail potential (Fig. 2 *B*), demonstrating that deactivation was voltage dependent and was faster than activation measured at the same voltages. These results suggest that during activation and deactivation, the channel may undergo different state transitions.

For voltage-gated channels, the holding potential determines the closed state in which the channel resides; the more depolarized the holding potential, the closer to the open state and the shorter the time to activate the channel (Cole and Moore, 1960). Therefore, the effect of holding potential on the time course of activation was examined. Fig. 3 *A* shows current traces recorded from a single oocyte at 40 mV from holding potentials varying from −120 to −60 mV. As the holding potential was made more positive, the delay associated with the onset of the current decreased. If the initial delay reflects the time required to transit closed states before opening, then the loss of the delay at more depolarized holding potentials may represent channels in closed states closer to the open state. Upon subsequent depolarization, the channels undergo fewer conformational transitions before opening. This interpretation is consistent with observations first made by Cole and Moore for the

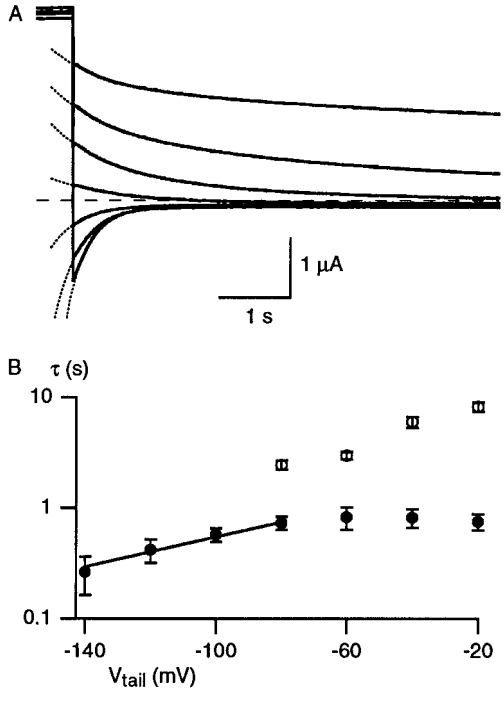

FIGURE 2 Deactivation kinetics of I_{sK}. (*A*) Tail currents were recorded during repolarization to potentials from −20 to −140 mV, in 20-mV increments, after activation of I_{sK} with a 20-s pulse to 40 mV. Time constants for deactivation were derived by fitting a double exponential to the tail currents (·····). For potentials less than −80 mV, a single exponential was used. (*B*) Time constants of deactivation plotted on a log scale as a function of tail potential. The data for the fast component of deactivation were fit with a single exponential yielding $2.6e^{V/64}$. Bars represent the standard deviation from five experiments.

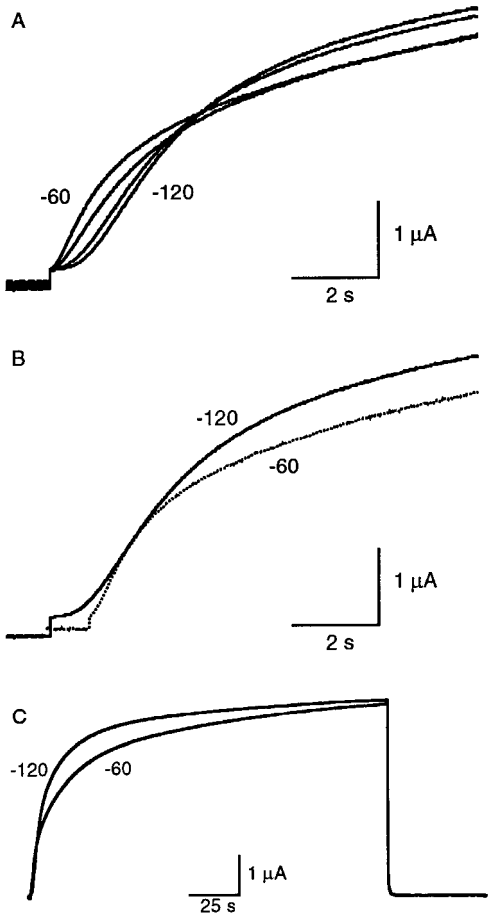

FIGURE 3 Effect of holding potential on activation of I_{sK}. (*A*) Currents elicited by step depolarization to 40 mV from holding potentials between −60 and −120 mV in 20-mV increments. (*B*) Comparison of currents elicited from a holding potential of −120 mV and −60 mV with the current trace recorded from a holding potential of −60 mV shifted by a delay of 0.91 s. (*C*) Long depolarization (3 min) to 40 mV from a holding potential of −120 and −60 mV.

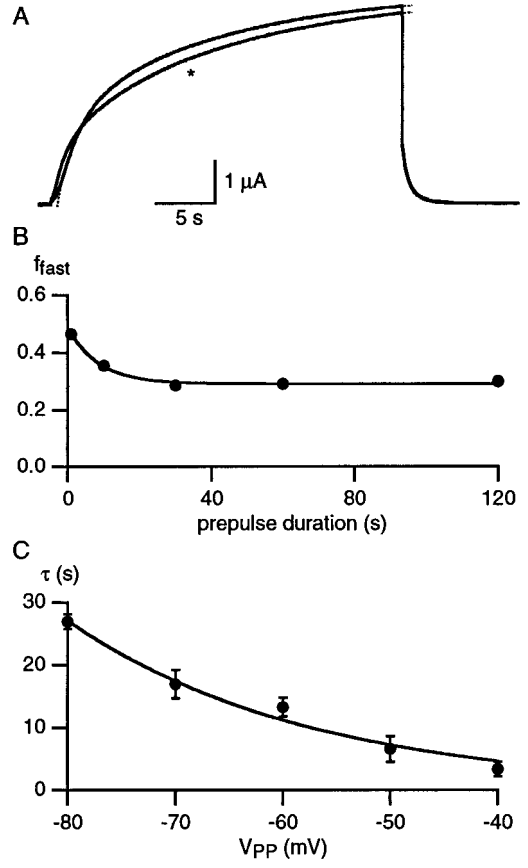

FIGURE 4 Effect of prepulse duration on activation of I_{sK}. (*A*) Currents elicited by depolarization to 40 mV from a holding potential of −60 (*) or −120 mV. The rising phase of the current was fit with a double exponential (·····). Average values for the fast and slow time constants were $\tau_{fast,-120}$ = 3.7 ± 0.4 s, $\tau_{fast,-60}$ = 3.6 ± 0.4 s, and $\tau_{slow,-120}$ = 14.7 ± 1.0 s, $\tau_{slow,-60}$ = 13.9 ± 1.0 s (n = 5). The relative amplitudes of the fast component, defined as $a_{fast}/(a_{fast} + a_{slow})$, where a_{fast} and a_{slow} are the amplitudes of the fast and slow component, respectively, were f_{fast} = 0.44 ± 0.02 at −120 mV and 0.28 ± 0.01 at −60 mV, $p < 0.05$ (n = 5). (*B*) Effect of prepulse duration to −60 mV on the relative amplitude of the fast component. The relative amplitude of the fast component, f_{fast}, determined from double-exponential fits as in *A*, was plotted as a function of the prepulse duration to −60 mV from a holding potential of −120 mV. The data were fit with a single exponential, yielding a time constant of 8.5 s (——). (*C*) Voltage dependence of transition between fast and slow components. The time constant for transition between the fast and slow components determined in *B* is plotted as a function of prepulse potential. Data are fit with a single exponential function, yielding 0.8 $\exp^{-V/23}$. Data represent mean ± standard deviation from five experiments.

squid giant axon potassium current (Cole and Moore, 1960). However, the Cole-Moore hypothesis predicts that if the gating mechanism involves sequential transitions through independent closed states, then the current traces obtained at a single test potential from different holding potentials should superimpose when shifted along the time axis to an extent equal to the decreased delay. However, the current traces presented in Fig. 3 *A* cross over each other and do not overlay when shifted along the time axis (Fig. 3 *B*), even though the steady-state currents are similar after longer pulses (Fig. 3 *C*). This finding suggests that I_{sK} gating cannot be explained by a simple sequential model of independent and identical, first-order transitions between closed states. An alternative explanation is that distinct activation pathways are used from different holding potentials.

To more closely examine the cross-over effect, currents were evoked from holding potentials of either −60 or −120 mV. The rising phase of the current traces obtained at 40 mV was fit with a double exponential (Fig. 4 *A*). The time constants of the fast and slow components were not statistically different between the two holding potentials, but the relative contribution of the fast component was greater at the more hyperpolarized holding potential (Fig. 4 *A*). A possible explanation is that gating of I_{sK} may proceed through kinetically distinct pathways, and the distribution of channels between pathways is determined by the potential before channel activation. To determine whether the effect of holding potential was time dependent, a prepulse protocol was used to evaluate the relative contribution of the fast component at different prepulse durations. From −120 mV,

a prepulse to −60 mV was applied for a varying period of time before a command to 40 mV. The rising phase of the current was fit with a double exponential, and the relative amplitude of the fast time constant was plotted as a function of the prepulse duration (Fig. 4 *B*). These data were well described by a single exponential, yielding a time constant of 13.3 ± 1.5 s for −60 mV. Similar experiments were performed for prepulse potentials between −80 and −40 mV. The time constants calculated for the different potentials were plotted as a function of the prepulse potential, and the data were fitted by a single exponential, demonstrating that the distribution between the two different pathways was voltage dependent (Fig. 4 *C*). To look at the voltage dependence of the reverse process, cells were held at −50 mV, and a prepulse to voltages between −100 and −60 mV was applied for varying amounts of time before a command to 40 mV. The reverse transition was similarly described by a voltage-dependent time constant (data not shown).

These results show that the potential before activation may partition the channel between one of two activation pathways. To evaluate transitions nearer the open state, experiments to measure reactivation after repolarization to potentials between −80 and −140 mV were performed (Fig. 5). The membrane was stepped to 40 mV for 15 s and then back to −140 mV (interpulse voltage) for varying durations (interpulse interval) before a return to 40 mV; the time course of reopening was assessed. Channel closure at −140 mV was relatively rapid (τ = 270 ms; Fig. 5 *A*). After a brief repolarization (<1 s), not all of the channel had closed, and upon reactivation some of the current was instantaneous, reflecting the residual open channels. Note that the currents became appreciably more sigmoidal, and the time course of reactivation became slower with interpulse intervals longer than deactivation. This suggests that when allowed to deactivate for longer times, the channel progresses to closed states more distant from the open state and must undergo multiple transitions before reopening. The kinetics were assessed by fitting a double exponential to the rising phase of reactivation. The time constant of the fast component increased with interpulse duration, whereas the time constant of the slow component did not change appreciably. The fast time constant was plotted as a function of the interpulse duration (Fig. 5 *C*), and the data points were fit with a single exponential, yielding a time constant of 1.5 s. This time constant reflects a recovery process that was significantly slower than deactivation of the tail current and represents a slower component in the closing transition. Similar experiments were performed for interpulse voltages from −140 to −80 mV, and the derived time constants were plotted as a function of the interpulse voltage. These data were fit with a single exponential, revealing the voltage dependence for the slow closing transition (Fig. 5 *D*).

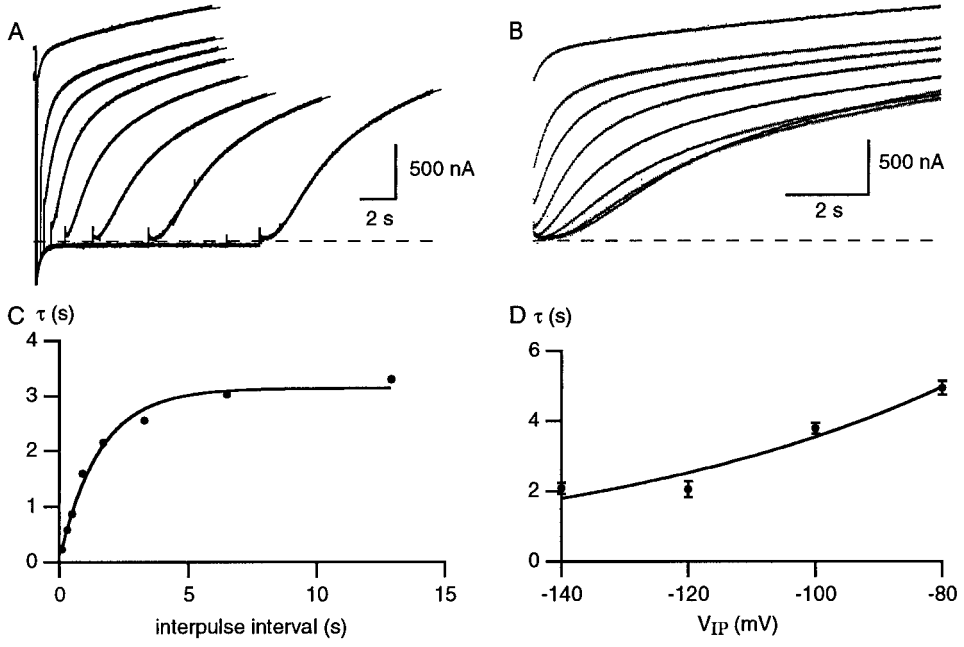

FIGURE 5 Reactivation of I_{sK}. (*A*) From a holding potential of −140 mV, the membrane was stepped to 40 mV for 15 s and then back to −140 mV (interpulse voltage) for varying durations (interpulse interval) before a return to 40 mV. The time constant for deactivation of the tail current at −140 mV was 0.27 s, and the time constant of the envelope of the instantaneous current on reactivation as a function of interpulse duration was 0.25 s. (*B*) Overlay of reactivation traces from *A*. (*C*) Time constant of the fast component of reactivation, τ_{fast}, determined from a double-exponential fit to the rising phase of the currents in *B*, plotted as a function of the interpulse interval. The data were fit with a single exponential, yielding a time constant of 1.56 s. (*D*) The time constant for the slow deactivation, as determined in *C*, was determined for interpulse voltages between −140 and −80 mV and plotted as a function of the interpulse voltage. Data represent the mean ± SD for five cells. The data were fit to a single-exponential function, yielding a voltage dependence for the time constant of the slow transition of 20 $\exp^{V/58.9}$.

Overlaying the currents during reactivation in Fig. 5 *A* showed that as the interpulse interval at −140 mV was increased, the lag before reactivation became more pronounced (Fig. 5 *B*). As for activation, the Cole-Moore hypothesis predicts that a gating mechanism with any number of independent and identical transitions will follow the same time course of reactivation, with a simple shift along the time axis, as the duration of the interpulse interval is varied. However, as for activation, reactivation of I_{sK} is inconsistent with Cole-Moore behavior; the traces do not overlay when shifted along the time axis.

Fig. 5 showed that transitions near the open state were extremely fast compared to activation from the most closed state. To assess the voltage dependence of this rapid transition near the open state, channels were activated by a 20-s pulse to 40 mV from a holding potential of −80 mV. Then a fraction of the channels (~73%; see Fig. 6 legend) were closed by a brief hyperpolarizing pulse to −140 mV, followed by depolarizing commands to test potentials between −40 and 40 mV (Fig. 6 *A*). The rising phase of reactivation was fit with a double exponential, and the fast time constant, which contributed to ~80% of the outward current at 40 mV, was plotted as a function of the test potential (Fig. 6 *B*). Fig. 6 *B* showed that the time constant of reactivation was only slightly voltage dependent between −40 and 40 mV. The brevity of the interpulse interval ensured that the channels reside in closed states close to the open state, and the lack of voltage dependence suggests a rate-limiting voltage-independent transition near the open state.

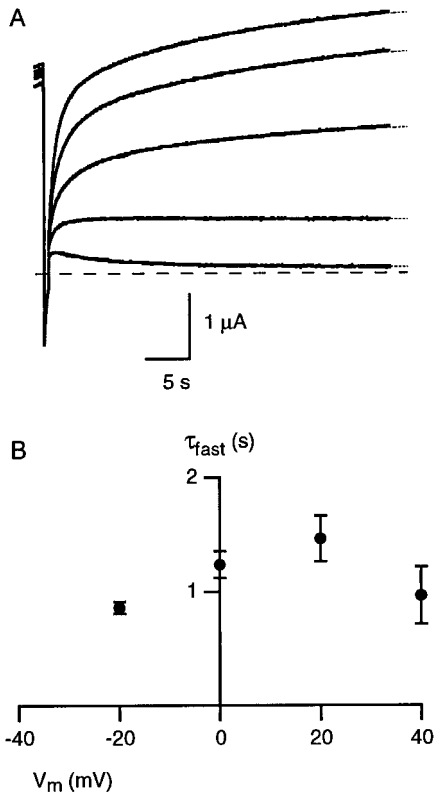

FIGURE 6 Voltage dependence of kinetics of reactivation. (*A*) I_{sK} was activated by a 20-s pulse to 40 mV from a holding potential of −80 mV. The membrane was briefly repolarized (500 ms) to −140 mV to close a fraction of the channels (~73% of the tail current deactivated) before reactivation to test potentials between −40 and 40 mV. (*B*) The voltage dependence of reactivation was examined by fitting the rising phase of reactivation with a double exponential, and the fast time constant, τ_{fast}, was plotted as a function of the test potential. Data represent the mean ± SD for five cells.

The results described above indicate that the gating of I_{sK} proceeds through closed states along kinetically distinct pathways before channel opening. To determine whether the channels may enter kinetically distinct open states, the effect of the duration of a test pulse on deactivation kinetics was examined. From a holding potential of either −120 or −60 mV, currents were evoked by depolarizing commands to 40 mV for varying durations (Fig. 7, *A* and *B*). From either holding potential, repolarization from 40 mV to −60 mV deactivated the channels with similar time courses, and the time constants of deactivation were plotted as a function pulse duration (Fig. 7, *C* and *D*). The similar time constants from either holding potential suggest that I_{sK} deactivate from either a single open state or kinetically indistinguishable open states.

Taken together, these results demonstrate that gating of I_{sK} cannot be explained by a sequential state model that involves identical and independent steps. Two different kinetic models have been proposed to explain the dependence of I_{sK} gating in *Xenopus* oocytes on the amount of mRNA injected (Cui et al., 1994). Although none of these models reproduced the deviation from the Cole-Moore prediction described in our study, inherent to the models is an interaction between subunits that modulates channel gating; a higher density of subunits increases this interaction.

DISCUSSION

In the experiments described here, we used ionic current measurements to examine the gating properties of I_{sK}. The main findings of this study are that the activation kinetics of I_{sK} depend on the holding potential and that reactivation is much faster than activation. These gating characteristics may permit I_{Ks} to reduce the risk of premature action potentials.

The I_{sK} channel is remarkable because its activation kinetics are so sensitive to the holding potential and to reactivation conditions. The dependence on holding potential and the fast reactivation kinetics may be of physiological significance in heart, as the coassembly of the minK protein with K_vLQT1 is thought to underlie the slow component of the delayed rectifier in cardiac ventricular myocytes. This current, I_{Ks}, is activated during the plateau of the cardiac action potential and contributes to repolarization of the membrane to the resting potential. However, brief and aberrant depolarizations (early "after-depolarizations") sometimes occur, which may trigger a premature action potential. Such premature action potentials, after the falling

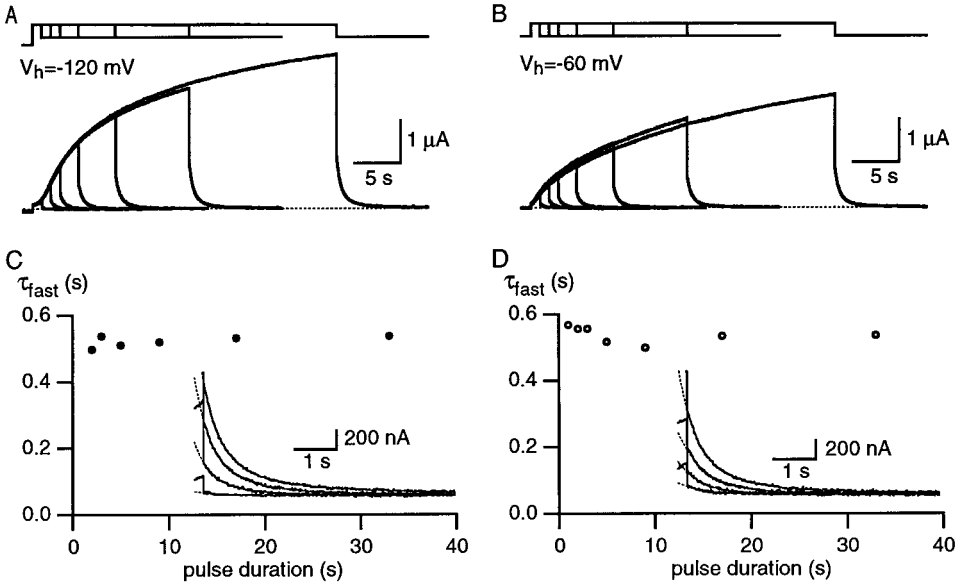

FIGURE 7 Deactivation as a function of pulse duration. (*A*) Currents (*bottom traces*) evoked by depolarizing commands to 40 mV for varying pulse durations, from a holding potential of −120 mV (*top traces*). Tail currents were measured on repolarization to −60 mV. (*B*) Currents evoked by depolarizing commands to 40 mV for varying pulse durations, from a holding potential of −60 mV. Tail currents were measured on repolarization to −60 mV. (*C*) The fast time constant of deactivation, plotted as a function of pulse duration for the depolarizations in *A*, initiated from a holding potential of −120 mV. (*Inset*) Superimposed tail currents for pulse durations of 1, 3, 9, and 33 s with associated double-exponential fits (*dotted traces*). (*D*) The fast time constant of deactivation, plotted as a function of pulse duration for the depolarizations in *B*, initiated from a holding potential of −60 mV. (*Inset*) Superimposed tail currents for pulse durations of 1, 3, 9, and 33 s with associated double-exponential fits (*dotted traces*).

phase of the previous cardiac action potential, are mirrored by the protocol used here to examine reactivation of I_{sK} (Fig. 6). Indeed, reactivation of the endogenous I_{Ks} in guinea pig ventricular myocytes after a 20-ms repolarization to −75 mV is ~0.73-fold faster than after 300-ms repolarization (Groh et al., 1997). Under these conditions, I_{Ks} activation is accelerated, providing a more rapidly activating outward current, which may reduce the risk of premature action potentials after an early after-depolarization.

Recent studies show that I_{Ks} channels are a heteromeric complex of minK and K_vLQT1 subunits. K_vLQT1 has structural features shared with other voltage-gated potassium channels, six membrane-spanning domains, a homologous pore region, and positively charged residues in the S4 segment. However, K_vLQT1 is not sufficient for the formation of I_{Ks} channels and requires minK (Sanguinetti et al., 1996; Barhanin et al., 1996). There is evidence that minK subunits contribute to the pore (Goldstein and Miller, 1991; Wang et al., 1996), and mutations within the transmembrane and C-terminal domains of minK affect voltage-dependent gating and regulation by protein kinase C, respectively (Busch et al., 1992; Varnum et al., 1993; Tzounopoulos et al., 1995), suggesting that minK subunits are involved in channel gating of I_{sK}. Consistent with this hypothesis, application of the cross-linking agent 3,3-dithiobis(sulfosuccinimidyl) proprionate locks I_{sK} channels in the open state and prevents deactivation upon repolarization (Varnum et al., 1995). It is possible that the unusual gating kinetics described in this report may reflect voltage-dependent subunit assembly involving either or both of the subunits that contribute to I_{sK} channels.

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