Single-Channel Properties of I\textsubscript{Ks} Potassium Channels

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ABSTRACT Expressed in *Xenopus* oocytes, KvLQT1 channel subunits yield a small, rapidly activating, voltage-dependent potassium conductance. When coexpressed with the minK gene product, a slowly activating and much larger potassium current results. Using fluctuation analysis and single-channel recordings, we have studied the currents formed by human KvLQT1 subunits alone and in conjunction with human or rat minK subunits. With low external K\textsuperscript{+}, the single-channel conductances of these three channel types are estimated to be 0.7, 4.5, and 6.5 pS, respectively, based on noise analysis at 20 kHz bandwidth of currents at +50 mV. Power spectra computed over the range 0.1 Hz–20 kHz show a weak frequency dependence, consistent with current interruptions occurring on a broad range of time scales. The broad spectrum causes the apparent single-channel current value to depend on the bandwidth of the recording, and is mirrored in very "flickery" single-channel events of the channels from coexpressed KvLQT1 and human minK subunits. The increase in macroscopic current due to the presence of the minK subunit is accounted for by the increased apparent single-channel conductance it confers on the expressed channels. The rat minK subunit also confers the property that the outward single-channel current is increased by external potassium ions.

KEY WORDS: KvLQT1 • long QT syndrome • fluctuation analysis • minK

INTRODUCTION

Expression of the minK protein is associated with potassium channel activity in a variety of tissues (Busch and Stuessbrich, 1997). The minK (also called I\textsubscript{Ks}) protein underlies a slowly activating current in uterine smooth muscle (Boyle et al., 1987) that is developmentally regulated; it also underlies the slow delayed rectifier current I\textsubscript{Ks} in cardiac tissue (Freeman and Kass, 1993; Varnum et al., 1993) and a potassium current in epithelial cells of the ear (Sakagami et al., 1991; Marcus and Shen, 1994). Expression of this small (129–130 amino acids) protein in heterologous systems yields at most a small potassium current whose magnitude saturates at low expression levels (Lesage et al., 1993; Blumenthal and Kaczmarek, 1994), suggesting that it must combine other subunit types to form functional I\textsubscript{Ks} channels. In the channel complex minK appears to be present in multiple copies (Tzounopoulos et al., 1995), quite possibly as few as two (Wang and Goldstein, 1995).

The other partner in the I\textsubscript{Ks} channel is the product of the LQT1 gene. Long QT syndrome (LQTS)\textsuperscript{1} is a genetically heterogeneous disorder that causes cardiac arrhythmias and leads to sudden death. One of several loci for this disorder, LQT1 is located on chromosome 11 (Keating et al., 1991) and is the gene for a potassium channel subunit named KvLQT1 (Wang et al., 1996b). Although KvLQT1 subunits produce a potassium current when expressed alone, much larger currents having the slow kinetic characteristics of I\textsubscript{Ks} are obtained from the coexpression of KvLQT1 and minK subunits (Barhanin et al., 1996; Sanguinetti et al., 1996; Yang et al., 1997). The LQTS-associated mutations in the KvLQT1 gene appear to reduce the expressed I\textsubscript{Ks} current in a dominant-negative fashion (Shalaby et al., 1997; van den Berg et al., 1997).

Because KvLQT1 subunits give rise to functional potassium channels when expressed alone, it is interesting to consider the nature of the interaction between minK and KvLQT1 that produces larger and more slowly activating currents when these genes are coexpressed. It has been argued that minK serves as a regulator of channel activity (Attali et al., 1993; Ben-Efraim et al., 1996), but evidence is accumulating that minK residues form part of the pore of the I\textsubscript{Ks} channel complex (Wang et al., 1996a; Sesti and Goldstein, 1998; Tai and Goldstein, 1998). In a recent study using COS cells (Romey et al., 1997), it was concluded that the effect of minK coexpression was greatly to increase channel number while decreasing the single-channel conductance of the channels expressed from KvLQT1 subunits. In the present study, we revisit the single-channel properties of the KvLQT1 and coexpressed channels, making use of fluctuation analysis and single-channel recordings from *Xenopus* oocytes. A companion study considers the single-channel properties of coexpressed

\textsuperscript{1}Abbreviation used in this paper: LQTS, long QT syndrome.
channels containing mutant minK subunits as well (Sesti and Goldstein, 1998).

Materials and Methods

DNA and RNA Synthesis

Human and synthetic rat minK cDNAs (Hausdorff et al., 1991; Goldstein and Miller, 1991) were obtained from Dr. S. Goldstein (Yale University) and propagated in pGEM-A and pBP2 vectors, respectively (Swanson et al., 1990; Tai and Goldstein, 1998). Point mutations in the minK constructs were made by PCR and verified by sequencing. Plasmids of rat and human minK were linearized with NotI and MluI, respectively. cRNAs were transcribed with the MEGAscript T7 and SP6 RNA polymerase kits (Ambion Inc., Austin, TX). Two human KvLQT1 constructs were obtained from Drs. M. Sanguinetti and M. Keating (University of Utah), which we call s-KvLQT1 and l-KvLQT1. The s-KvLQT1 (Sanguinetti et al., 1996) has a truncated NH2 terminus, while l-KvLQT1 is full-length, having 95 additional residues at the NH2 terminus (Yang et al., 1997). Each KvLQT1 gene was subcloned into a modified Bluescript vector (Bluescript KSM; gift from W. Joiner, Yale University) that incorporates β-globin untranslated sequences and a poly-A tail for increased protein translation in oocytes. Plasmids of s-KvLQT1 and l-KvLQT1 were linearized with NotI and XbaI, respectively, and transcribed with the MEGAscript T3 RNA polymerase kit (Ambion Inc.). Sizes of transcribed cRNAs were verified by gel electrophoresis.

Electrophysiology

Human KvLQT1 cRNA (5.8 ng) was injected into Xenopus oocytes alone or in conjunction with 1 ng minK cRNA. We use the notation hI_K to denote channels resulting from coexpression of human minK and human KvLQT1, rhI_K to denote channels from the combination of rat minK and hKvLQT1, and I_LQT to denote channels expressed from hKvLQT1 alone. In this study, only the full-length l-KvLQT1 variant was used for expressing hI_K and I_LQT channels; most rhI_K recordings were made with this variant as well. The rhI_K channels formed with the truncated s-KvLQT1 construct had identical behavior in terms of voltage dependence and single channel unitary current.

Patch- and voltage-clamp recordings were done at room temperature, 7-12 d after RNA injection. Patch clamp recordings were obtained using EPC-9 (HEKA Electronic, Lambrecht, Germany) or Axopatch 200B (Axon Instruments, Foster City, CA) amplifiers. Pipettes were pulled from 7052 glass (Corning Glass Works, Corning, NY) to a tip size of 2–5 μm. Pipettes with tip diameters of ~30 μm were used for recording I_LQT channel currents. These pipettes were pulled from thin-walled borosilicate capillaries (PG165T; Warner Instruments, Hamden, CT). The standard bath solution for patch clamp recordings was (mM) 7 KCl, 93 K-aspartate, 1 EGTA, and 10 HEPES. The standard pipette solution was (mM) 0.2 KCl, 100 NaCl, 1 MgCl2, 1.8 CaCl2, and 10 HEPES. All solutions were titrated to pH 7.4.

For two-microelectrode voltage clamp recordings, an OC-725 amplifier (Warner Instruments) was driven by the Pulse software (HEKA Electronic) and an analogue interface (ITC-16; Instrument Corp., Mineola, NY). Microelectrodes were filled with 1 M KCl and had 0.1–0.3 MΩ resistance. The standard bath solution for voltage clamp recordings, denoted ND96, contained (mM) 96 NaCl, 2 KCl, 0.1 CaCl2, 1 MgCl2, and 5 HEPES, pH 7.4.

Half-amplitude threshold analysis (Colquhoun and Sigworth, 1995) was used to idealize single-channel recordings for kinetic analysis and the reconstruction of ensemble time courses. For noise analysis, the macroscopic currents induced by a series of depolarizing pulses were recorded on video tape using a VR-10 Digital Data Recorder (Instrutech Corp.). Data were then transferred digitally from tape through the VR-10 Digital Recorder using the program VCatch developed in our laboratory. The raw data (94 kHz sampling rate) were filtered and decimated using a digital Gaussian filter. Power spectra were calculated from data decimated and filtered to 10 Hz, 100 Hz, 1 kHz, and 10 kHz bandwidths. A power spectrum covering the frequency range 0.1 Hz–20 kHz was obtained by combining the four individual spectra after correcting for filter responses.

Statistical quantities are expressed as mean ± SEM with the number of determinations n ≥ 3 unless otherwise stated.

Results

Activation of I_K and I_LQT Channels

In oocytes co-injected with minK and KvLQT1 cRNAs, both cell-attached and inside-out patch recordings showed slowly activating outward currents with monotonically increasing noise during 5-s depolarizations (Fig. 1, A and B). As characterized by Boltzmann fits to isochronal conductance–voltage curves, the voltage dependence of activation of these hI_K and rhI_K channels is quite shallow, with an effective charge of 1–1.2 e0 and a midpoint voltage near +55 mV. The main difference in behavior between the two channel types is the more rapid time course of activation in the hI_K channels. Both channel types show gradually increasing current even at the end of 60-s depolarizations to +50 mV (Fig. 1, D and E).

For comparison, the activation of currents resulting from the injection of hKvLQT1 cRNA alone is shown from a cell-attached giant patch recording in Fig. 1 C. The fragility of the giant patches precluded recordings at large positive voltages, but a Boltzmann fit over the accessible voltage range yields a half-activation voltage of −6 mV, considerably more negative than that of I_K channels and consistent with previous observations (Sanguinetti et al., 1996). The tail currents show a “hook” in the time course, characteristic of KvLQT1 currents (Sanguinetti et al., 1996; Pusch et al., 1998). The patch recordings shown in Fig. 1 all have similar kinetics and voltage dependence to the corresponding whole cell-currents obtained by two-electrode voltage clamp.

Reversal potentials of rhI_K currents were measured from macroscopic tail currents obtained in inside-out or cell-attached patch recordings with 100 mM K+, Na+, Rb+, or Cs+ in the pipette; in each case, the bath solution contained 100 mM K+. Table I (top) shows the reversal potentials of the rhI_K channels. The table also gives the computed permeability ratios. The permeability ratios are very similar to those obtained from voltage clamp recordings of oocytes injected with rat minK RNA (Hausdorff et al., 1991). Like many other potassium channels, the permeability sequence is K+ > Rb+ > Cs+ > Na+.

It was more difficult to obtain patch recordings with macroscopic hI_K currents. Therefore, the ion selectiv-
ity of these channels was characterized from whole cell currents with 100 mM K\textsuperscript{+}, Na\textsuperscript{+}, Rb\textsuperscript{+}, or Cs\textsuperscript{+} in the bath solution. For comparison, the reversal potentials of rhIK\textsubscript{s} channels were also measured in this way, using the same batch of oocytes. There was no significant difference in reversal potentials between these two channel types (Table I).

**Single hIK\textsubscript{s} Channel Current**

If we assume that the IK\textsubscript{s} channel only has one conductance level with unitary current \(i\), then for \(n\) channels the variance of current fluctuations will depend on the mean current \(I\) according to

\[
\sigma^2 = Ii - \frac{I^2}{n}
\]

(Sigworth, 1980). We shall denote by \(\hat{i}\) an estimate of \(i\) obtained from fitting Eq. 1 to the variance–mean relationship. For this analysis, a series of current sweeps was collected by applying repetitive depolarizing pulses to \(-50\) to \(+110\) mV in 20-mV steps from a \(-80\)-mV holding potential. No leak subtraction, filtered at 500 Hz. The isochronal voltage dependence of normalized conductance (bottom) was fitted with a Boltzmann function \(G = G_{\text{max}}/(1 + \exp[(V_{1/2} - V)/k])\), with \(V_{1/2} = 52\) mV and \(k = 20\) mV. (B) Activation of rhIK\textsubscript{s} currents from an inside-out patch recording with 130 mM K-aspartate, 10 mM KCl, 1 mM EGTA, pH 7.4 in the bath; 100 mM NaCl, 0.2 mM KCl, 1 mM MgCl\textsubscript{2}, 1.8 mM CaCl\textsubscript{2}, pH 7.4 in the pipette. Current activation (top) at \(-70\) to \(+150\) mV in 20-mV steps from \(-80\)-mV holding potential. Leak current was subtracted by the P/5 protocol with \(-100\)-mV leak holding potential; data were filtered at 500 Hz. The isochronal voltage dependence of rhIK\textsubscript{s} from three patches is fitted by a Boltzmann function with \(V_{1/2} = 56\) mV and \(k = 26\) mV. (C) Activation of I\textsubscript{LQT} from a giant patch (30-μm diameter pipette tip) with 93 mM K-aspartate, 7 mM KCl, 1 mM EGTA, pH 7.4 in the bath, 96 mM NaCl, 2 mM KCl, 1 mM MgCl\textsubscript{2}, 0.1 mM CaCl\textsubscript{2}, pH 7.4 in the pipette. Currents were induced by depolarizing pulses from a \(-80\)-mV holding potential to potentials of \(-70\) to \(+60\) mV in 10-mV steps, and repolarization to \(-60\) mV; filtered at 100 Hz. No leak current correction was applied. Normalized peak conductance was fitted with \(V_{1/2} = -6\) mV and \(k = 18\) mV. Conductance in all three cases was computed assuming a linear open-channel current–voltage relationship with a reversal potential of \(-80\) mV. (D) hIK\textsubscript{s} current from a cell-attached patch recording with standard solutions, showing the response to a 60-s depolarization to \(+50\) mV. Filter bandwidth 40 Hz. (E) A corresponding recording of rhIK\textsubscript{s} current.
Table I
Reversal Potential $V_r$ of $I_{Ks}$ Channels with Various External Ions

| Conditions                  | $K^+$  | $Rb^+$ | $Cs^+$  | $Na^+$  |
|-----------------------------|--------|--------|---------|---------|
| rhIks patch clamp ($n = 3$) | $-1.5 \pm 0.5$ | $-7.3 \pm 0.6$ | $-73 \pm 0.7$ | $<-110$ |
| rhIks voltage clamp ($n = 6$) | $-1.2 \pm 0.6$ | $-11 \pm 0.4$ | $-61 \pm 0.3$ | $-110 \pm 1.2$ |
| hIks voltage clamp ($n = 9$) | $-1.2 \pm 0.2$ | $-10 \pm 0.2$ | $-60 \pm 0.4$ | $-108 \pm 0.7$ |
| rhIks $P_x/P_k$ (patch, $n = 3$) | $1$ | $0.75 \pm 0.03$ | $0.06 \pm 0.003$ | $<0.01 \pm 0.002$ |

Reversal potentials (in millivolts) were estimated from inside-out or cell-attached patch clamp recordings or from two-electrode voltage clamp. The pipette solution contained 100 mM of test cation $X$; bath solution contained 100 mM K$^+$. For voltage clamp measurements, reversal potentials were obtained using 100 mM of the test cation in the bath solution. The relative permeability of rhIks to ion $X$ (bottom row) was calculated as $P_x/P_k = \exp(\Delta V_r/RT)$, where $\Delta V_r = V_r(X) - V_r(K)$, as obtained from patch recordings.

bandwidth. The discrepancy between the two estimates of unitary current suggests that a substantial amount of variance is contained in high-frequency components.

To investigate the high-frequency components of the hIks current fluctuations, we computed the power spectrum of the macroscopic currents. Pairs of aligned current traces were subtracted as shown in Fig. 2D. Power spectra were computed from subtracted traces (Sigworth, 1981) by fast Fourier transform and the resulting power spectrum after correction for background noise is shown in Fig. 2E. It has a remarkably straight 1/f dependence over five decades of frequency. The weak frequency dependence of the spectral density implies that the observed noise variance will be heavily dependent on filter cutoff frequency. From Parseval’s theorem, we have

$$S^2 = \int \hat{S}(f) \hat{H}(f)^2 df,$$

where $S(f)$ is the power spectral density of the current fluctuations and $\hat{H}(f)$ is the filter transfer function. To give an idea of the effect of filter bandwidth, the spectral density in Fig. 2E was integrated numerically and converted into unitary current amplitude according to the expression

$$i_s(f) = \mathcal{T}^{-1} \int_0^f S(f') df'.$$

where $\mathcal{T}$ is the time-averaged mean current and $i_s(f)$ is the apparent unitary current at bandwidth $f$. As can be seen in Fig. 2E, $i_s$ increases strongly with filter bandwidth, and is still increasing at $f = 20$ kHz. Thus, fluctuation analysis is expected to yield any of a variety of unitary current amplitudes, depending on the bandwidth. At 20 kHz, $i_s$ is 0.47 pA at +50 mV.

The expression in Eq. 3 is missing a correction term (Sigworth, 1981) and therefore underestimates the unitary current by a factor of about $1 - p$, where $p$ is the mean open probability. Thus, the apparent unitary currents from spectral analysis (Fig. 2F) of 0.2 and 0.47 pA, at 100 Hz and 20 kHz bandwidth, respectively, become ~0.25 and 0.6 pA when $p = 0.2$ is assumed. These values agree with those obtained from the mean–variance analysis (Fig. 2B and C).

Unitary currents roughly 0.5 pA in size should be visible in single-channel recordings. Obtaining single-channel patches was difficult, however, because the hIks channels appeared to be highly clustered in the oocyte membrane so that patches typically contained either tens of channels or no channels at all. The distribution of patch current density was very broad, as determined from more than 100 patches (Fig. 3). Fig. 4A shows one of our best candidates for an hIks single-channel current. This sweep was recorded from a multiple-channel patch but appears to have only one channel active. As would be expected from the very broad power spectrum of macroscopic current fluctuations, the channel current shows very rapid flickering. From recordings at three voltages, the single-channel conductance is estimated to be 3 pS at 200 Hz bandwidth (Fig. 4C).

Conductance of $I_{LQT}$ Channels

Injection of the KvLQT1 cRNA alone results in small $K^+$ currents having more rapid kinetics than hIks channels. Might the smaller current result from smaller single-channel currents? In the case of the hIks channels, mean–variance and spectral fluctuation analysis yielded reasonable estimates of the unitary current at +50 mV, comparable to what was observed in a patch recording. To determine the $I_{LQT}$ unitary current, we used the same fluctuation-analysis methods and similar experimental protocols. The only difference was that in attempting to record those currents, we encountered a very low channel density. Using pipettes with 2–5-μm tip diameters, we saw no current in 12 patches from oocytes having mean whole-cell currents of 4 nA. Therefore, we used much larger pipettes (30-μm tip diameter) to obtain macroscopic channel currents. Fig. 5A shows one cell-attached giant patch having a mean current of 240 pA at +50 mV. This recording shows the characteristic “hook” of outward tail current that is seen in $I_{LQT}$ channels. The mean–variance relationship, computed from data filtered at 200 Hz, is poorly fitted
by the parabolic function of Eq. 1; however, linear fits are consistent with unitary currents of 0.03–0.04 pA, as is shown in Fig. 5 C.

Spectral analysis of the fluctuations was also performed. The spectrum shows several discernible components, and can be well fitted by the sum of three Lorentzian functions (Fig. 5 E). The integral of the spectrum, scaled to show the apparent unitary current, shows \( i_s \) increasing with bandwidth but possibly reaching a limiting value of ~0.09 pA at 20 kHz. The unitary current is therefore about one fifth of that of the hIKs channels. We were not able to obtain any convincing single-channel recordings of this current.

**Figure 2.** Fluctuation analysis of hIKs currents. (A) Mean current and time-dependent variance computed from 30 sweeps filtered at 100 Hz. The current was induced by +50-mV depolarizing pulses from a -80 mV holding potential in a cell-attached patch recording. Pulses were delivered every 32 s. (B) Mean–variance plot of 100 Hz–filtered currents. A fit of Eq. 1 yields unitary current \( i_v = 0.28 \) pA and number of channels \( n = 368 \); the corresponding apparent open probability \( P_{\text{max}} = 0.43 \). (C) Mean–variance plot from a total of 20 sweeps at 10 kHz bandwidth from the same patch. In this case, 124 depolarizations to +50 mV were delivered every 32 s. The fit yields \( i_v = 0.51 \) and \( P_{\text{max}} = 0.24 \). (D) A pair of successive current traces and their difference filtered at 1 kHz; same set of data as in A. (E) The top trace is the resulting power spectrum of currents from 30 sweeps after correction for background noise. The contribution \( S_{\text{n}} \) from ion transport during channels opening was estimated according to \( S_{\text{n}} = 2eI = 1.4 \times 10^{-29} \text{A}^2/\text{Hz} \) and was subtracted. The solid line indicates a power-law fit \( S/f = 10^{-24}/f^{0.9} \text{A}^2/\text{Hz} \). The mean current was 45 pA, and the unitary current estimate \( i_v = 0.47 \) pA at 20 kHz bandwidth. The lower trace shows the spectrum (values plotted one decade lower in the graph for clarity) computed from another representative data set, where the mean current was 39 pA; the unitary current \( i_v = 0.44 \) pA at 20 kHz. (F) Dependence of apparent unitary current \( i_s \) (Eq. 3) on filter cut-off frequency at +50 mV. Variance was calculated from the numeric integral of the power spectrum over the frequency range 0.1 Hz–10 kHz.

**Single Channel Properties of rhIKs Channels**

We also studied channels formed by coexpression of rat minK with human KvLQT1 subunits. The currents from these channels (Fig. 1) show similar noise properties and voltage dependence to those containing human minK subunits. Fig. 6 shows the fluctuation analysis of these channels. The power spectrum (Fig. 6 B) does not have the simple power-law frequency dependence of the hIKs channels, but can be fitted by one \( 1/f \) component plus several Lorentzian components, where a minimum of four Lorentzians was required for a good fit. The presence of discernible Lorentzians suggests that rhIKs channels may have more clearly distin-
kinds of ionic current respond with a lower threshold than hIKs channels. The unitary current, i, was calculated from the power spectrum from this experiment. Again, i shows a strong dependence on bandwidth, as shown in Fig. 6 C, with an estimated unitary current of 0.67 pA at 20 kHz. Depending on the estimated open probability, this value should be increased somewhat, for example to 0.84 pA assuming p = 0.2.

The mean–variance analysis was also applied to same set of data. The fit of Eq. 1 to the mean–variance plot (Fig. 6 E) yields an estimate of the unitary current i of \( \sim 0.28 \) pA at 100 Hz bandwidth. This is similar to the value obtained from hIKs channels at this bandwidth.

The presence of discernible Lorentzian components in the power spectrum suggests that the rhIKs channels should show less flickering than the hIKs channels. Patch recordings indeed showed single-channel events, but as was the case with hIKs channels, of >200 trials, we were unable to obtain a one-channel recording of sufficient duration to allow kinetic analysis. Shown in Fig. 7 is a recording from a patch containing three channels using pulses to +50 mV. Channels open after a latency of a few seconds, often first to a subconductance level before reaching the full single-channel current (Fig. 7 B). To verify that these channel events correspond to the macroscopic currents, we computed the channel open probability from the idealizations of 60 sweeps. It has a slowly activating time course that reaches an open probability of 0.45 at the end of the 5-s depolarization. This time course superimposes well on the time course of current in a multichannel patch (Fig. 7 C). The time course of IKs current is shown in Fig. 6 D.
course of activation can be described by the distribution of first latencies to channel opening. Ignoring the subconductance levels, we measured the first latency to the fully open state and corrected it for the presence of three channels (Aldrich et al., 1983). When scaled by the factor 0.8, it matches very well the time course of the open probability. This correspondence is consistent with the idea that, once a channel opens, it remains open with a substantial probability (apparently 0.8 at this time resolution).

Slow variations in single-channel activity were observed in this patch, with occasional null sweeps (Fig. 7 B, middle) occurring throughout the recording (Fig. 7 F). A subsequent recording at +20 mV from the same patch (Fig. 8) shows similar kinetic behavior of the single channels. Again, subconductance levels are sometimes seen to precede the full opening of the channel (Fig. 8 A) and the slow time course of activation is explained by long first latencies (Fig. 8 B). At this smaller depolarization, a higher frequency of null sweeps was seen (Fig. 8 C).

External Potassium Dependence of IKs Channels
The conductance and gating of some types of K+ channels depend on external K+ concentration. We tested
the external potassium dependence of whole-cell currents using continuous bath perfusion. Fig. 9 shows currents at +30 mV as the bath solution was switched between 0.2 and 10 mM K\(^+\). The hIKs and rhIKs channels have opposite responses to the change in external potassium. Switching from 0.2 to 10 mM K\(^+\) reduces the hIKs current by 20%, an effect that can be explained by the decrease in driving force; however, there is a 20% current increase under the same conditions with rhIKs channels. That higher external potassium increases outward current was also observed in the I\(_{Kr}\) current through HERG channels (Sanguinetti et al., 1995). The effect of external K\(^+\) was also tested for ILQT channels (Fig. 9 C). In this case, the currents were smaller when external K\(^+\) was increased. These experiments show that coexpression with the rat minK gene product changes the sensitivity the I\(_{LQT}\) channels to external potassium.

A comparison of the human and rat minK sequences (Murai et al., 1989; Fig. 9 D) shows many differences in the extracellular (NH\(_2\)-terminal) and intracellular (COOH-terminal) regions, but only one nonconserved residue in the putative transmembrane domain. As a first attempt to locate the region responsible for the differences in K\(^+\) sensitivity, we made complementary mutations at this position. The resulting constructs, human (V47I) and rat (I48V) minK were coexpressed with human KvLQT1 and the external potassium sensitivity was...
assayed by the ratio of peak current in 10 mM external K\(^+\) to that in 0.2 mM K\(^+\) (Table II). The mutation in human minK had no significant effect on the K\(^+\) sensitivity, but, with the rat mutation increased, K\(^+\) significantly decreased the current (P < 0.01), which is the opposite effect to that seen with the wild-type rat minK subunit. Thus, this residue in the membrane-spanning region appears to contribute to the external potassium sensitivity.

Because the single-channel rhIK\(_s\) currents can be resolved in patch-clamp recordings, it should be possible to examine the origin of the increase in outward current in these channels when extracellular potassium concentration is increased. Fig. 10 A shows three representative single-channel currents from inside-out patches at +50 mV and filtered at 100 Hz. The currents were estimated to be 0.56, 0.44, and 0.37 pA with [K]\(_o\) equal to 10, 0.2, and 0 mM. The single-channel current is seen to increase when [K]\(_o\) increases, even as the driving force decreases. Recordings from six patches show increased conductance over the voltage range of −30 to +80 mV with higher [K]\(_o\) (Fig. 10 B). The [K]\(_o\) dependence of conduc-
tance appears to saturate above 2 mM, and its magnitude accounts for all of the increase in macroscopic current observed on raising extracellular potassium in the case of the rhIK\(_s\) channel.

**Discussion**

This study has considered the single-channel properties of I\(_{Ks}\) channels obtained from the coexpression of the human or rat minK protein with human KvLQT1, and has compared these properties with the expression of KvLQT1 subunits alone in *Xenopus* oocytes. We conclude that the I\(_{Ks}\) channels have a higher single-channel conductance than channels from KvLQT1 alone, and that the sensitivity to external potassium ions is reflected in the size of single-channel currents in the case of the rhIK\(_s\) hybrid channel. The I\(_{Ks}\) single-channel currents are roughly 0.6 pA at +50 mV. The relatively low conductance of these slowly activating channels might be important to reduce membrane potential fluctuations in cells where I\(_{Ks}\) serves to shape long-duration action potentials.

![Figure 7](image-url)
Size of Single-Channel Currents

The rapid flickering of currents in these channels makes difficult the determination of the single-open-channel current. When fluctuation analysis is used to estimate the single-channel current, the bandwidth of the recording must be sufficient to capture the fastest fluctuations, or else the variance will be underestimated, providing an underestimate of the single-channel current. The direct observation of single-channel currents suffers from a similar limitation: if a channel’s current contains many brief interruptions, a single-channel recording at low bandwidth will show a reduced apparent single-channel current and increased apparent open probability. The very noisy appearance of the hIKs recording at 500 Hz (Fig. 4 A) suggests that this bandwidth is not sufficient to resolve the true open-channel current. Fluctuation analysis allows a wider range of frequencies to be explored.

A two-state channel with opening and closing rate constants $\alpha$ and $\beta$ yields current fluctuations having a Lorentzian power spectrum with a corner frequency $f_c = 1/2\pi(\alpha + \beta)$. Above $f_c$, the Lorentzian decays with frequency as $f^{-2}$; this relatively rapid decay means that the observed variance of the fluctuations converges rapidly to the correct value as the bandwidth is increased above $f_c$. On the other hand, no convergence results in the case of an $f^{-1}$ frequency dependence, like that shown in Fig. 2 E for the hIKs channels. Such a frequency dependence results in an observed variance that increases without limit as the bandwidth increases. Because bandwidth is related to the time scale of measurement, one could speak of an effective single-channel current value that depends on the time scale under which it is measured. Channels having stable open and closed states, such that the power spectrum of fluctuations from these channels decay rapidly at higher frequen-
cies, show a distinct single-channel current value given sufficient recording bandwidth. For the hIKs channel, however, the 20-kHz limit of our power spectrum measurements was not sufficient to reach this regime. Thus we do not know the asymptotic value of the variance; we also do not know the exact open probability value that is necessary to correct the estimate of the single-channel current. The variance computed from fluctuations up to 20 kHz (Fig. 2), when corrected for an estimated absolute open probability of $z_{0.2}$, result in the estimated single-channel current of 0.6 pA at $150 \text{ mV}$, or a chord conductance of $z_{4.5} \text{ pS}$. These values are consistent with the current extremes observed in single-channel recordings (Fig. 4).

Another way to summarize the problem posed by the hIKs channel is that the very rapid current fluctuations make it difficult experimentally to distinguish, on the basis of time scales, between gating or channel-block phenomena on the one hand and the ion conduction process on the other. The apparent single-channel conductance values are influenced by the very rapid interruptions in the channel current.

The channels formed by hKvLQT1 subunits expressed alone or in combination with rat minK subunits showed less extreme behavior. Although the spectra of the current fluctuations are also very broad, they are not as featureless as those of hIKs currents and can be fitted by multiple Lorentzian components. Limiting single-channel current values at $150 \text{ mV}$ of 0.09 and 0.84 pA are obtained from fluctuation analysis. These correspond to conductances of $z_{0.7}$ and 6.5 pS. The rhIKs channels resulting from coexpression were also

### Table II

| Channels | hIKs | rIKs | LQT | hIKs (V471) | rIKs (I48V) |
|----------|------|------|-----|------------|-------------|
| $r$, mean ± SD | 0.72 ± 0.08 | 1.16 ± 0.07 | 0.67 ± 0.15 | 0.78 ± 0.12 | 0.91 ± 0.07 |
| Oocytes tested | 6 | 8 | 8 | 3 | 8 |

The parameter $r = I_{\text{peak}}(10 \text{ K})/I_{\text{peak}}(0.2 \text{ K})$ is the ratio of currents measured at the end of 5-s depolarizations to $+50 \text{ mV}$ with either 0.2- or 10-mM external potassium solutions bathing the oocyte. Oocytes were injected with the given constructs and currents were measured using the two-electrode voltage clamp.

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Figure 9. Effect of extracellular potassium on peak current of hIKs, rhIKs, and LQT in the two-electrode voltage clamp. (A) Magnitude of hIKs current at the end of 5-s depolarizations to $+30 \text{ mV}$ as the bath solution was switched between 0.2 and 10 mM K$. The lower panel shows current traces corresponding to the times indicated at top. (B) rhIKs current; (C) LQT current; (D) the amino acid sequences of human and rat minK in the vicinity of the putative transmembrane region (box).
observed directly from single-channel recording at 100 Hz bandwidth. There they appeared to have single-channel currents at 150 mV of 0.5 pA, depending on extracellular K\textsuperscript{+} concentration (Fig. 10).

In native tissues, the cardiac I\textsubscript{Ks} current has been seen to have small fluctuations. Walsh et al. (1991) estimated unitary conductances of 1, 3.2, and 4.8 pS for 0, 0.2, and 10 mM external K\textsuperscript{+}, respectively. (C) External potassium dependence of single channel conductance. Error bars represent SEM (n = 2 for 0 mM and n = 4 for 10 mM; the points at 0.2 and 2 mM K\textsuperscript{+} are single observations). The superimposed fit is the function $g = 1.8 + 2.8/(1 + 0.5 \text{mM}/[K])$ pS.

A similar argument can be made concerning the possible heterogeneity of channel types. When \textit{minK} and \textit{KvLQT1} cRNAs are co-injected, it is possible that hybrid channels are expressed having various stoichiometries, and the fluctuation analysis will give a weighted-average value. Again, it should be kept in mind that larger channel currents make larger contributions to the variance, and therefore predominate in the weighted average. Thus, if our coinjections produced a variety of channel types, the estimated conductance probably reflects the largest conductance value. Further, the good correspondence between the fluctuation analysis of rhI\textsubscript{Ks} currents and direct single-channel recordings argues that heterogeneity in channel conductances is not a serious problem.

A similar question was raised by \textit{minK} coexpression experiments. Romey et al. (1997) addressed this question through single-channel recordings and noise analysis of expressed currents in COS cells. They concluded that the addition of \textit{minK} subunits to \textit{KvLQT1} channels caused a reduction of single-channel conductance from 7.6 to 0.6 pS. To account for the increase in macroscopic current, they conclude that coexpression with \textit{minK} causes the channel density to increase by a large factor, some 60-fold. Our studies of these channels in \textit{Xenopus} oocytes lead to the opposite conclusion, that a large part of the observed current increase on coexpression of \textit{minK} arises from a large increase in conductance, and have also used the assumption that there is only one nonzero conductance level. It is likely that one or more of these assumptions is false. Evidence has been presented by Pusch et al. (1998) that \textit{KvLQT1} channels have two open states, and we see clear subconductance levels in single-channel recordings of the rhI\textsubscript{Ks} channels (Figs. 7 B and 8 A). If there are multiple conductance levels, the estimated single-channel current will lie between the largest and smallest single-channel current, and will depend on the probabilities of occupancy of the various conductance states. It should be kept in mind, however, that the high-conductance states tend to dominate the estimated conductance, because the contribution of a state’s current $i$ to the variance is proportional to $i^2$. Thus, our single-channel conductance estimates are likely to approximate the values for the largest conducting states.

**How Coexpression of \textit{minK} Affects \textit{KvLQT1} Current**

Expression of \textit{KvLQT1} subunits produces small, rapidly activating potassium currents; coexpression of these with \textit{minK} results in slowly activating I\textsubscript{Ks} currents that are several-fold larger. These differences in the expressed currents have been seen in a variety of expression systems, including \textit{Xenopus} oocytes, Sf9 cells, and in the mammalian cell lines CHO and COS (Barhanin et al., 1996; Sanguinetti et al., 1996; Romey et al., 1997). Is the increase in \textit{KvLQT1} current on coexpression with \textit{minK} due to an increase in channel density or an increase in the single-channel current? Romey et al. (1997) addressed this question through single-channel recordings and noise analysis of expressed currents in COS cells. They concluded that the addition of \textit{minK} subunits to \textit{KvLQT1} channels caused a reduction of single-channel conductance from 7.6 to 0.6 pS. To account for the increase in macroscopic current, they conclude that coexpression with \textit{minK} causes the channel density to increase by a large factor, some 60-fold.
from an increase in single-channel conductance. From fluctuation analysis, we estimate a single-channel conductance of \( \sim 0.7 \) pS for KvLQT1 channels. We estimate the conductance of human \( I_{Ks} \) channels to be \( \sim 4.5 \) pS. The discrepancy between our results and those of Romey et al. (1997) might be explained by the difference between COS cell and oocyte expression systems. This, however, is unlikely because the behavior of the channels is similar in the various systems; further, Romey et al. (1997) report the same single-channel conductance value for \( I_{Ks} \) channels expressed in Xenopus oocytes as in COS cells.

Our results disagree with this previous work in two respects. First, we obtain a larger single-channel conductance for the human \( I_{Ks} \) channels than reported by Romey et al. (1997). This can be explained largely by the frequency dependence of fluctuations in this channel. Our value of 4.5 pS is based on fluctuation analysis at 20 kHz and on single-channel recordings at 500 Hz bandwidth; their value of 0.6 pS was based on fluctuation analysis at a bandwidth of 150 Hz under similar ionic conditions. Our conductance estimate of 6.5 pS for the closely related rh\( I_{Ks} \) channel (Figs. 6–10), for which openings are more readily resolved, supports the higher conductance estimate.

The other disagreement concerns the conductance of channels arising from the expression of KvLQT1 subunits alone. Romey et al. (1997) found well-resolved single-channel events in COS cells having a conductance of 7.6 pS. In our macropatch recordings, we find a remarkably noiseless current (Fig. 5). The power spectrum from the macropatch recording shows a broad frequency dependence, with a limiting conductance value of \( \sim 0.7 \) pS apparently being reached at 20 kHz bandwidth. There is always the danger that the currents in patch-clamp recordings are not properly identified, such that unitary events from one channel type are ascribed to another. Although we have not performed a pharmacological identification of our currents, we note that the kinetics of activation and the tail currents in our macropatch recordings agree very well with the currents observed from KvLQT1 channels in whole oocytes and in other expression systems (Fig. 1 C, Barhanin et al., 1996; Sanguinetti et al., 1996; Romey et al., 1997), supporting the view that it is these channels whose fluctuations we have measured.

Our results agree well with those of Sesti and Goldstein (1998), who studied channels expressed from KvLQT1 subunits alone and with human minK. They used symmetrical 100 mM potassium solutions and thereby obtained higher conductance values (4 and 16 pS) compared with ours (0.7 and 4.5 pS). Under the different ionic conditions, the single-channel outward currents are expected to be similar at large depolarizations. At \( \pm 50 \) mV and 20 kHz bandwidth, our estimate for the single-channel current of \( I_{Ks} \) channels is 0.6 \( \pm 0.2 \) pA; here the error bounds reflect an estimate of statistical and systematic errors in the fluctuation analysis used. The corresponding estimate at 25 kHz bandwidth given by Sesti and Goldstein (1998) is 0.8 \( \pm 0.2 \) pA.

### Kinetics of \( I_{Ks} \) Channels

In addition to rapid flickering, the currents through single \( I_{Ks} \) channels show slow gating processes. At depolarizations to \( \pm 20 \) and \( \pm 50 \) mV (Figs. 7 and 8), the main determinant of the activation time course is seen to be the latency to first channel opening. In the rh\( I_{Ks} \) channels where single-channel events could be readily resolved, dwells in a subconductance state were often seen to precede full channel opening. The rh\( I_{Ks} \) channel activity also waxes and wanes on a time scale of \( \sim 30 \) s, as seen by groups of successive blank sweeps in patch recordings (Figs. 7 and 8).

### The Conductance of minK “Channels”

The discovery that KvLQT1 subunits coassemble with the minK gene product to produce the \( I_{Ks} \) current (Barhanin et al., 1996; Sanguinetti et al., 1996) has clarified some of the puzzling aspects of the “\( I_{minK} \) current” that is seen in Xenopus oocytes when minK is expressed alone (Busch and Suessbrich, 1997). It is now clear that this current results from the combination of minK with an endogenous, Xenopus KvLQT1 homologue that is expressed at low levels (Sanguinetti et al., 1996). The difficulty that we and others have had in attempting to define the single-channel characteristics of \( I_{minK} \) are now understandable in view of the difficulties we have encountered in recording from single \( I_{Ks} \) channels. In a preliminary communication (Yang and Sigworth, 1995), we reported fluctuation analysis of a slowly activating current seen in patches from Xenopus oocytes, but many subsequent attempts were unsuccessful to establish this current as the same as the macroscopic, potassium-selective \( I_{minK} \). It is possible that our patch currents, which from fluctuation analysis had a unitary current value below 1 fA, were contaminated with currents from an endogenous channel or ion transporter having slow kinetics, similar perhaps to the transporter studied by Schlief and Heinemann (1995).

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We thank W.N. Joiner for the Bluescript-KSM vector and Y. Yan for cRNA preparation and oocyte injection. We also thank Dr. S.A.N. Goldstein for minK cDNA, and Drs. M. Sanguinetti and M. Keating for the human KvLQT1 cDNA.

Original version received 30 July 1998 and accepted version received 21 October 1998.
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Due to a printing error, Eqs. 2 and 3 were printed incompletely. They should appear as follows:

\[ \sigma^2 = \int_0^\infty S(f) |H(f)|^2 df \]  \hspace{1cm} (2)

\[ i_s(f) = \int_0^f S(f') df' \]  \hspace{1cm} (3)