Temperature dependence of early and late currents in human cardiac wild-type and long Q-TΔKPQNa\(^+\) channels

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Nagatomo, Toshihisa, Zheng Fan, Bin Ye, Gayle S. Tonkovich, Craig T. January, John W. Kyle, and Jonathan C. Makielski. Temperature dependence of early and late currents in human cardiac wild-type and long Q-TΔKPQNa\(^+\) channels. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H2016–H2024, 1998.—Na\(^+\) current (I\(_{\text{Na}}\)) through wild-type human heart Na\(^+\) channels (hH1) is important for normal cardiac excitability and conduction, and it participates in the control of repolarization and refractoriness. I\(_{\text{Na}}\) kinetics depend strongly on temperature, but I\(_{\text{Na}}\) for hH1 has been studied previously only at room temperature. We characterized early I\(_{\text{Na}}\) (the peak and initial decay) and late I\(_{\text{Na}}\) of the wild-type hH1 channel and a mutant channel (ΔKPQ) associated with congenital long Q-T syndrome. Channels were stably transfected in HEK-293 cells and studied at 23 and 33°C using whole cell patch clamp. Activation and inactivation kinetics for early I\(_{\text{Na}}\) were twofold faster at higher temperatures for both channels and shifted activation and steady-state inactivation in the positive direction, especially for ΔKPQ. For early I\(_{\text{Na}}\) (<24 ms), ΔKPQ decayed faster than the wild type for voltages negative to −20 mV but slower for more positive voltages, suggesting a reduced voltage dependence of fast inactivation. Late I\(_{\text{Na}}\) at 240 ms was significantly greater for ΔKPQ than for the wild type at both temperatures. The majority of late I\(_{\text{Na}}\) for ΔKPQ was not persistent; rather, it decayed slowly, and this late component exhibited slower recovery from inactivation compared with peak I\(_{\text{Na}}\). Additional kinetic changes for early and peak I\(_{\text{Na}}\) for ΔKPQ compared with the wild type at both temperatures were 1) reduced voltage dependence of steady-state inactivation with no difference in midpoint, 2) positive shift for activation kinetics, and 3) more rapid recovery from inactivation. This study represents the first description of human Na\(^+\) channel kinetics near physiological temperature and also demonstrates complex gating changes in the ΔKPQ that are present at 33°C and that may underlie the electrophysiological and clinical phenotype of congenital long Q-T Na\(^+\) channel syndromes.

long Q-T syndrome; human heart; ion channels; sodium current

SODIUM ION CURRENT (I\(_{\text{Na}}\)) is important for cardiac excitability and conduction through effects on the action potential upstroke, and late I\(_{\text{Na}}\) is also involved in maintaining the action potential plateau. These effects in turn play important roles in the mechanisms of arrhythmia by reentry and triggered automaticity. I\(_{\text{Na}}\) has generally been studied at less than physiological temperatures for convenience and to reduce and slow the currents sufficiently for adequate voltage control. The few studies of nonhuman mammalian cardiac I\(_{\text{Na}}\) at higher temperature show a steep and sometimes complex temperature dependence that causes, for example, a shift in the inactivation relationship (2, 16, 17).

The congenital long Q-T syndrome (LQT) is a hereditary cardiac disorder that causes syncope and sudden death from ventricular arrhythmias such as torsades de pointes and ventricular fibrillation. LQT3 is one form of the disease for which the defective gene has been identified as SCN5A, which encodes the voltage-dependent cardiac Na\(^+\) channel α-subunit (hH1; see Refs. 8, 12, 23, 24). Deletion of three amino acids (ΔKPQ: lysine, proline, and glutamine) at positions 1505–1507 in intracellular linker between domain III and domain IV is one of the mutants in LQT3 (23, 24). Altered current decay rates and a small "persistent" current at depolarized potentials have been reported for the ΔKPQ (1, 3, 6, 22). These studies have reported diverse results for the voltage dependence of current decay rates and the magnitude of late currents, both of which are presumed to be important in the arrhythmogenic mechanism that results in the clinical phenotype. No studies have reported the recovery characteristics of the late current. Moreover, just as with the wild-type human Na\(^+\) channel, all previous reports of ΔKPQ kinetics were obtained at room temperature. It is not known whether or not the kinetic effects of ΔKPQ are the same at more physiological temperatures.

We characterized gating kinetics of the wild-type channel and ΔKPQ hH1Na\(^+\) at room temperature and at 33°C, comparing 1) kinetics of early macroscopic I\(_{\text{Na}}\) decay, 2) amplitude and decay of late I\(_{\text{Na}}\), 3) steady-state inactivation and activation of peak I\(_{\text{Na}}\), and 4) recovery from inactivation of peak and late I\(_{\text{Na}}\). Some of these data have been reported previously in abstract form (18).

MATERIALS AND METHODS

Clones and construction of ΔKPQ mutation. The human heart Na\(^+\) channel done that we used was kindly provided by Dr. H. Hartmann (Baylor College of Medicine, Houston, TX). This channel is designated hH1a because it differs in nine amino acids with one deletion from the channel reported as hH1 (9): V120I, A130G, R552A, T559A, H987Q, Q1027R, W1085G, R1087E, G1088A, deletion Q1077. The differences are not known to cause functional effects; therefore, for simplicity we refer to the channel as hH1. The nucleotide and amino acid numbering follow Hartmann et al. (9).

The ΔKPQ mutation was made by polymerase chain reaction (PCR) techniques according to Higuchi et al. (10). The primers used generated PCR products through the Kpn I (base pair 4253) and Bst E II (base pair 4657) sites of hH1a. The mutant primers deleted bases 4539 through 4547, which codes for amino acids lysine (K1504), proline (P1505), and...
glutamine (Q1506). In addition, these primers introduced a silent restriction site by a C-to-A mutation at base 4532, which adds a BamHI site without altering the amino acid sequence. The PCR products were subcloned into hH1a in the pGEM3 construct at Kpn I and BstE II, and expression was first tested in Xenopus oocytes. The mutant region was then shuttled to hH1a in a mammalian expression vector Rz/CMV (Invitrogen) using unique Age I (base pair 1045) and BstE II (base pair 4657) sites. The entire PCR generated region was completely sequenced and confirmed the deletion and also confirmed that no other unwanted changes were made in the channel.

Cell preparation and transfection. Approximately $5 \times 10^5$ cells from a transformed human embryo kidney cell line (HEK-293) were seeded on a 60-mm-diameter plate (Falcon 3001) with 3 ml of culture medium a day before the transfection. Culture medium was MEM complete medium containing MEM (Eagle's salts and L-glutamine), 10% fetal bovine serum (FBS), 2 mM L-glutamine, 0.1 mM MEM nonessential amino acid solution, 1 mM MEM pyruvate solution, 10,000 units penicillin, and 10,000 g streptomycin.

Transfection was carried out by using a cationic liposome method. The cationic lipid used was N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl sulfate (DOTAP) at a concentration of 1 µg/ml. DOTAP was obtained from the Medical School Vector Core Laboratory, University of Wisconsin-Madison. DOTAP (20 µg) and plasmid DNA (5 µg) were diluted with Opti-MEM (GIBCO-BRL), and final volume for transfection was 200 µl. The DOTAP-DNA mixture was incubated with cells for 5 h in the 2 ml of Opti-MEM medium. After incubation, cells with the DOTAP-DNA mixture were replaced with 3 ml of normal culture medium. To select stably transfected cells, geneticin (G418 sulfate; GIBCO-BRL) at a total concentration of 800 µg/ml was added for ~15 days, at which time surviving single colonies were isolated and cultured with 400 µg/ml geneticin for 1–3 wk. Cells were then treated with a trypsin-EDTA solution (0.25% trypsin, 1 mM EDTA; GIBCO-BRL), centrifuged at 2,000 rpm for 2 min, and frozen in FBS (GIBCO-BRL) with 7% DMSO for storage up to 6 mo. Cells for study were thawed, transferred to normal culture media for 1–6 h, and then transferred directly to the experimental chambers.

Electrophysiological recordings. Macroscopic $I_{\text{Na}}$ was recorded using the whole cell patch-clamp technique. The bath (extracellular) solution contained (in mM) 140 NaCl, 4 KCl, 1.8 CaCl$_2$, 0.75 MgCl$_2$, and 5 HEPES (pH 7.4 with NaOH). The pipette solution contained (in mM) 120 CsF, 20 CsCl, 5 EGTA, and 5 HEPES (pH 7.4 set with CsOH). The electrodes were pulled (P-87; Sutter Instrument) from borosilicate glass and heat polished with a microforge (MF-83) to a final resistance of $<1.2 \text{ M} \Omega$ when filled with the electrode solution. Cells for study were placed in a Plexiglas chamber with continuous-flowing bath solution mounted on an inverted microscope (Nikon) in a Faraday cage. The temperature was controlled via heat exchange through a water jacket surrounding the bath solution, and the bath temperature was monitored. Electrophysiological recordings were carried out at room temperature of 23°C and at 33°C. Experiments were attempted at 37°C, but the patch-clamp seal did not remain stable for a time sufficient to complete the protocols. Membrane currents were recorded with an Axopatch 200 amplifier (Axon Instruments). Data were acquired using pCLAMP v6.03. Data were digitized at 100 kHz for early current or 10 kHz for late current and were low pass filtered at 10 kHz. Holding potential was $-150 \text{ mV}$ except where otherwise noted as in, for example, recovery from inactivation protocols.

We observed a time-dependent shift of the steady-state inactivation relationship even though the preparation was otherwise stable. To minimize effects of the time-dependent change of availability, we recorded over a 5-min period beginning 2 min after the whole cell configuration was achieved. Steady-state availability was assessed initially and at the end of this period, and the shift was usually $<3 \text{ mV}$. In those cases in which the shift was $>3 \text{ mV}$ the data were discarded.

Data analysis. Passive leak subtraction of peak and late currents was performed by subtracting a linear leak extrapolated from holding currents at subthreshold potentials (less than $-100 \text{ mV}$) to the potential of interest (15). Saxitoxin (STX)-sensitive currents were measured by subtracting a trace obtained in the presence of 5 µM STX, a concentration that completely blocked $I_{\text{Na}}$, from a trace obtained earlier in the absence of STX (referred to as STX subtraction). Data were fit to model equations using nonlinear regression using pCLAMP v6.03 or SigmaPlot 3.0. Goodness of fit was judged both visually and by the sum of squared errors. In choosing models with a greater number of parameters to fit the data, as in a two-exponential fit over a single exponential fit, an F-ratio test was used ($P < 0.05$) to account for the increased number of free parameters. Mean data are expressed with their SE. All determinations of statistical significance of mean data were performed by using a Student’s t-test for comparisons of two means. A P value of $<0.05$ was considered statistically significant.

Technical considerations. Mammalian cell lines such as HEK-293 cells are tools widely used in studying structure-function relationships of ion channels, but this use is based on the supposition that endogenous currents are absent or minimal. Endogenous currents were measured in nontransfected HEK-293 cells and also in cells transfected with hH1 and the ΔKPQ where $I_{\text{Na}}$ was blocked by STX. Under our study conditions (CsF in pipette solution) a noninactivating outwardly rectifying current was measured at voltages $>0 \text{ mV}$. This current was between 100 and 500 pA and activated over several hundred milliseconds during a depolarizing step. This contrasts with a previous report in these cells of a rapidly activating and inactivating outward endogenous current under other conditions (KCl in pipette solution; see Ref. 27) and emphasizes the need to consider endogenous currents under the specific study conditions. The endogenous current also gradually increased over the data collection period; therefore, STX subtraction did not perfectly correct for the endogenous current. At voltages $<0 \text{ mV}$, the range of primary interest, the endogenous current was negligible ($<1\%$ of late $I_{\text{Na}}$ at 240 ms) but tended to increase with length of depolarization.

Adequate voltage control was achieved by using low-resistance pipettes, high conductive solutions, and series resistance compensation. To further minimize possible errors arising from voltage control, we selected donal lines expressing lower $I_{\text{Na}}$ density. The small size and low capacitance ($<10 \text{ pF}$) of these cells allowed for very rapid charging of the membrane capacitance. Two indexes of voltage control that we required to be present were a graded slope of the activation curve (Boltzmann slope factor $>5.0$) and scaling of currents of different amplitudes to the same test potential as in steady-state inactivation and recovery protocols. These indirect measures are standard, and they have been validated in other preparations (15). The data at higher temperatures where the currents are larger and faster showed no differences in slope of the activation relationship (Table 1), suggesting that voltage control was maintained.
Characterization of macroscopic currents. The activation relationship for \( I_{\text{Na}} \) was obtained from peak \( I_{\text{Na}} \) resulting from a 24-ms step to various test potentials from a holding potential of \(-150\) mV. Figure 1A shows representative current tracings for the wild-type current and the \( \Delta I_{\text{KPQ}} \) at 23 and 33°C from different cells. At the higher temperatures, macroscopic currents peaked earlier and decayed faster, and peak \( I_{\text{Na}} \) was greater. The \( \Delta I_{\text{KPQ}} \) showed faster initial decay compared with the wild-type current, and the peak of the current-voltage relationship was shifted in the positive direction (Fig. 1B).

To compare the differences in the time course of current activation and decay, representative currents for test potentials of \(-40, -20, 0, \) and \( 30 \) mV were normalized to their peak values, and the traces for wild-type current and the \( \Delta I_{\text{KPQ}} \) were superimposed (Fig. 2). Current decays were more rapid for the \( \Delta I_{\text{KPQ}} \) than the wild-type current at \(-40 \) and \(-20 \) mV, but, at 30 mV, current decays were more rapid for the wild-type current. Currents recorded at the higher temperature (Fig. 2, bottom) showed more rapid kinetics, but the differences between \( I_{\text{Na}} \) decay rates for \( \Delta I_{\text{KPQ}} \) and the wild type remained.

Figure 3 shows summary data for the voltage dependence of peak \( I_{\text{Na}} \) amplitude for \( I_{\text{Na}} \), time constants of \( I_{\text{Na}} \) decay, and fractional amplitude of \( I_{\text{Na}} \) decay at 23 and 33°C. Time to peak \( I_{\text{Na}} \) for an index of activation rate, was reduced at higher temperatures by about one-half (compare Fig. 3, A and B). \( I_{\text{Na}} \) for the \( \Delta I_{\text{KPQ}} \) peaked earlier than the wild type at \(-50 \) mV but tended to peak later at more depolarized potentials, and the crossover point was temperature dependent. \( I_{\text{Na}} \) decay, an indicator of inactivation rate, at most potentials was best fit to two exponentials over 24 ms, and the time constant for the fast component is shown in Fig. 3, C and D. Like time to peak, decay time constants were faster at the higher temperature. The first component of the time constants for macroscopic current decay was significantly \((P < 0.05)\) more rapid for the \( \Delta I_{\text{KPQ}} \) than for the wild-type current from \(-40 \) to \(-10 \) mV but crossed over at potentials more positive than 0 mV (Fig. 3C) at 23°C and at \(-15 \) mV (Fig. 3D) at 33°C. The difference in \( I_{\text{Na}} \) decay for the \( \Delta I_{\text{KPQ}} \) compared with the wild-type current at 33°C, however, was less than that at 23°C and did not reach statistical significance. This finding, therefore, may be of biophysical importance but less interesting for physiological or pathophysiological mechanisms.

Late \( I_{\text{Na}} \). Late \( I_{\text{Na}} \) was investigated using prolonged (240-ms) depolarizing steps to various test potentials from a holding potential of \(-150\) mV. The \( \Delta I_{\text{KPQ}} \) showed a readily apparent late inward \( I_{\text{Na}} \) (called persistent, “plateau,” or “pedestal” \( I_{\text{Na}} \) previously) at both 23 and 33°C (Fig. 4A). We measured \( I_{\text{Na}} \) at 240 ms and normalized it by dividing by the maximum peak amplitude of \( I_{\text{Na}} \) from the current-voltage relationship. The mean values of normalized late \( I_{\text{Na}} \) at 240 ms for the wild-type current were \(0.21 \pm 0.02\%\) at 23°C \((n = 15 \) experiments) and \(0.19 \pm 0.03\%\) at 33°C \((n = 8 \) experiments), and values of normalized late \( I_{\text{Na}} \) for the \( \Delta I_{\text{KPQ}} \) were \(0.76 \pm 0.06\%\) at 23°C \((n = 22 \) experiments).
and $0.60 \pm 0.10\%$ at $33^\circ$C ($n = 13$ experiments). The $\Delta$KpQ exhibited significantly more current at $240$ ms than the wild type at both $23$ and $33^\circ$C ($P < 0.01$), but there was no significant difference between $23$ and $33^\circ$C.

A large component of the late $I_{Na}$ for the $\Delta$KpQ was not persistent but decayed in a multiexponential manner (Fig. 4A). This decay was faster at $33^\circ$C compared with $23^\circ$C (Fig. 4A). To verify that these late currents were $I_{Na}$, we used STX, a relatively specific blocker of Na$^+$ channels. Representative $I_{Na}$ traces for the $\Delta$KpQ in response to a depolarization to $-20$ mV without STX (Fig. 4B, left) and with STX subtraction (Fig. 4B, right) confirm the late currents as $I_{Na}$. Data for the current-voltage relationship of leak-subtracted late currents measured at $20$, $40$, $120$, $240$, $480$, and $720$ ms are shown without STX (Fig. 4C, left) and with STX subtraction (Fig. 4C, right), indicating that late $I_{Na}$ was STX sensitive and decayed at all potentials.

To further characterize the decay of late $I_{Na}$, we fit $I_{Na}$ traces over $240$ ms with a three-component exponential function for the $\Delta$KpQ, and the results of the fitting are shown in Fig. 3. E and F, for time constants of decay and in Fig. 3, G and H, for the relative amplitudes of the corresponding components as well as the “baseline” or persistent current. Only two component results are shown for wild-type late $I_{Na}$, because three exponential fits were not consistently found, perhaps because of the smaller size of the late $I_{Na}$ in the wild type. At $23^\circ$C both $\Delta$KpQ and the wild-type current showed a second slow decay time constant between $3$ and $8$ ms with a modest voltage dependence (Fig. 3E), and this time constant was decreased by about one-half at $33^\circ$C (Fig. 3F). A third decay constant between $70$ and $100$ ms was detected for $\Delta$KpQ, again increasing modestly with stronger depolarizations (Fig. 3E). The time constant for this third component of decay, however, was not as strongly temperature dependent (Fig. 3F). Figure 3, G and H, shows the relative amplitude of each component of decay and the baseline or persistent current from the fit. For both wild-type and $\Delta$KpQ the relative amplitude of the fastest decay component was comparable at $23^\circ$C (Fig. 3G) and $33^\circ$C (Fig. 3H). The second or intermediate slow component amplitude was greater for the wild-type current than for $\Delta$KpQ at both $23^\circ$C (Fig. 3G) and $33^\circ$C (Fig. 3H), perhaps because the third component and baseline were too small to be distinguished in the wild-type current and were included in this component for the wild-type but not for $\Delta$KpQ. At $23^\circ$C the relative amplitude of the third component of decay for $\Delta$KpQ and the baseline component was comparable at or near $1\%$ of peak $I_{Na}$ (Fig. 3G), but at $33^\circ$C the baseline current was smaller, and the third component was larger (Fig. 3H).

Activation and steady-state inactivation. The voltage dependence of steady-state inactivation was assessed by a two-pulse protocol as indicated in the protocol diagrams in Fig. 5. In sets. Peak $I_{Na}$ was measured at a test potential of $-20$ mV after $1$-s-long conditioning potentials between $-150$ and $-30$ mV. Peak currents were normalized to the largest peak current obtained, and data were fitted with a Boltzmann function to yield a midpoint and slope factor. Table 1 shows the parameters of Boltzmann fits at $23$ and $33^\circ$C. The midpoint for availability was not significantly different between the wild-type current and the $\Delta$KpQ, although the slope factor was significantly different for $\Delta$KpQ at $23^\circ$C. There were no significant differences in either the slope factor or the midpoint between the wild-type current and the $\Delta$KpQ at $33^\circ$C.

The voltage dependence of activation was evaluated as a normalized peak conductance-voltage relationship (Fig. 5) and was fitted with a Boltzmann function. The midpoint of the activation relationship for the $\Delta$KpQ was shifted in the positive direction at $23^\circ$C ($\pm 0.05$) without a significant difference in slope factor. At $33^\circ$C, both the slope factor and midpoint between the wild-type current and the $\Delta$KpQ were significantly different (Table 1). As for the effects of temperature on the activation and inactivation kinetics, at $33^\circ$C both the inactivation and the activation curves for both channels tended to shift in the positive direction compared with $23^\circ$C although a significant difference was observed only for the $\Delta$KpQ (Table 1).

Recovery from inactivation of peak and late $I_{Na}$. Recovery from inactivation at $-120$ mV was studied with a two-pulse protocol (see Fig. 6, inset). Cells were stepped to $-20$ mV for $1$ s during which time $I_{Na}$ was
inactivated almost completely, and test pulses to −20 mV were delivered after recovery steps to −120 mV. Recovery from inactivation was best fit to a double exponential function, and the fast time constant was significantly faster in the ΔKPQ at 23°C. At 33°C, recovery from inactivation was more rapid than that at 23°C, and both the fast and the slow time constants were significantly faster in the ΔKPQ (Table 2). Interestingly, although the time constants themselves indicated that recovery was faster at higher temperature, the relative contribution of the slow component of the recovery process was increased at the expense of the fast component in both the wild-type current and the ΔKPQ at the higher temperature (see Table 2).

We also studied the recovery from inactivation of the late component of I\textsubscript{\textsc{Na}} to determine whether or not it recovered differently from the peak I\textsubscript{\textsc{Na}}. The same protocol as for peak I\textsubscript{\textsc{Na}} was used (1-s conditioning step to −20 mV, see Fig. 6, inset), but a prolonged test potential was used, and the I\textsubscript{\textsc{Na}} at 240 ms was measured and plotted for the recovery time (Fig. 6). Approximately 40% of late I\textsubscript{\textsc{Na}} at 240 ms did not inactivate with a 1-s conditioning pulse, corresponding to the proportion of baseline current noted previously (Fig. 3, G

Fig. 3. Voltage dependence of time to peak (A and B), fast time constant (τ\textsubscript{f}; C and D), slow time constant (τ\textsubscript{s}; E and F), and fractional amplitude of current decay (G and H) at 23 and 33°C. A-D: cells with comparable mean peak current amplitudes were chosen to help control for any effects that might arise from imperfect voltage control. Time-to-peak amplitudes of I\textsubscript{\textsc{Na}} (A and B) and the fast time constants of current decay (C and D) for WT (●) and ΔKPQ (○) are plotted against the test potential (V\textsubscript{t}) used to elicit I\textsubscript{\textsc{Na}}. Time to peak is the time from the onset of depolarization to peak I\textsubscript{\textsc{Na}}. To obtain decay rates and components, the portion of the trace from 90% of peak I\textsubscript{\textsc{Na}} to 24 ms was fit with a sum of exponentials (exp): I\textsubscript{\textsc{Na}}(t) = 1 − (A\textsubscript{1} × exp−t/t\textsubscript{1} + A\textsubscript{2} × exp−t/t\textsubscript{2} + A\textsubscript{3} × exp−t/t\textsubscript{3}) + offset, where \( t \) is time, and A\textsubscript{1} and A\textsubscript{2} are fractional amplitudes of fast and slow components, respectively. For 23°C, n = 5 (WT) and n = 6 (ΔKPQ) experiments and for 33°C, n = 5 (WT) and n = 5 (ΔKPQ) experiments. E and F: slow component of current decay for the ΔKPQ (second: ◻; third: □) at 23°C (n = 8 experiments) and at 33°C (n = 4 experiments). The portion of the trace between 90% of peak I\textsubscript{\textsc{Na}} and 240 ms was fit with three exponential functions: I\textsubscript{\textsc{Na}}(t) = 1 − (A\textsubscript{1} × exp−t/t\textsubscript{1} + A\textsubscript{2} × exp−t/t\textsubscript{2} + A\textsubscript{3} × exp−t/t\textsubscript{3}) + offset, where t\textsubscript{1–3} are time constants of first, second, and third components, respectively, and A\textsubscript{1–3} are fractional amplitudes of each component. Time constant of second component for WT with fitting over 24-ms trace is shown for reference (dotted lines). G and H: fractional amplitudes for WT (first: ●; second: ▲) and ΔKPQ (first: ○; second: △; third: □; offset: ◦) are plotted. For 23°C, n = 5 (WT) and n = 8 (ΔKPQ) experiments and for 33°C, n = 5 (WT) and n = 4 (ΔKPQ) experiments. Symbols represent means, and bars represent SE. *Statistically significant differences for WT vs. ΔKPQ.
and F). Late $I_{\text{Na}}$ for $\Delta$KPQ recovered more slowly from inactivation compared with peak $I_{\text{Na}}$, and the recovery process was more rapid at 33 than at 23°C.

**DISCUSSION**

Temperature dependence of human heart $I_{\text{Na}}$. This study is the first report of human cardiac $I_{\text{Na}}$ at near physiological temperatures (technical considerations limited the study to 33°C rather than 37°C). In general, the temperature dependence of the gating kinetics for the human heart Na⁺ channel is much like that reported for nonhuman cardiac channels (13, 14, 17) with a temperature coefficient for indexes of activation and inactivation of about two for both the wild type and the $\Delta$KPQ and a shift in activation. An interesting finding noted only briefly previously (14) is that the relative amplitude of the slow component of recovery is increased at higher temperatures for both the wild-type current and the $\Delta$KPQ. This implies that entry into the kinetic state(s) from which recovery is slow has a higher temperature dependence than entry into the more rapid recovery state(s). Both steady-state inactivation and activation relationships were more positive with increasing temperature in both the wild-type current and the $\Delta$KPQ. This observation has also been reported by Murray et al. (17) for guinea pig ventricular cells; the mechanism for this positive shift is unknown but is probably related to a different temperature dependence of the individual gating transitions. Repolarization of the action potential depends on a delicate balance of relatively small inward and outward currents during the cardiac action potential plateau. As computer modeling is increasingly used to explore the underlying arrhythmogenic mechanism (13, 26), including details of these kinetic differences with different isoforms, mutants, and temperature will be important in understanding the relative contributions of each current to the arrhythmia.
Comparison of the wild type and ΔKPQ kinetics. The ΔKPQ mutation lies in the III-I/IV linker thought to be the inactivation gate, and defective inactivation and persistent current have been postulated as the cause of Q-T prolongation (7, 9, 25). In the present study, however, we found complex effects of the ΔKPQ mutation on kinetics: 1) initial current decay was actually more rapid and less voltage dependent, that is, faster for negative test potentials and slower for positive test potentials; 2) late currents for ΔKPQ were larger than for the wild-type current but smaller than reported previously by other investigators; 3) late currents were not time independent but rather inactivated slowly with a multiexponential time course and recovered from inactivation with slower time course compared with peak \( I_{\text{Na}} \); 4) activation was shifted to more positive voltages; 5) the midpoint of steady-state inactivation was not different, but slope factor was changed at 23°C; and 6) recovery from inactivation was accelerated.

The present study showed that currents for ΔKPQ actually have a more rapid initial decay at most negative voltages, but a lower voltage dependence results in a crossover (Figs. 2 and 3) such that at positive voltages decay is faster for the wild type. Wang et al. (22) reported that the fast time constant of the ΔKPQ was larger than the wild type at potentials greater than −20 mV for a holding potential of −120 mV, but their data also clearly indicate a lesser voltage dependence of the fast time constant of the ΔKPQ. An et al. (1) reported that the onset of inactivation, using a holding potential of −90 mV, was faster for the ΔKPQ, but current decay was fitted by a single exponential function. A recent report by Chandra et al. (4) for channels expressed in mammalian cells showed that early decays fit by a single exponential were more rapid for ΔKPQ at negative voltages, results very similar to Fig. 3C. Other reports (3, 6) using the oocyte expression system showed faster current decay for ΔKPQ at test potentials of −20 or −10 mV. Taken together, these observations suggest that macroscopic current decay for the ΔKPQ has lower voltage dependence compared with the wild-type current and decay is faster at negative voltages. A positive shift of the activation curve for ΔKPQ compared with the wild-type current agrees with the data by Wang et al. (22). However, this kinetic change should not necessarily be attributed to a mutation-induced change in activation kinetics, because the faster initial rate decay might account for some of this change.

A major difference of our findings from previous reports was that persistent current was much less than that reported previously. An et al. (1) and Wang et al. (22) found that persistent currents were 4.0 and 2.6% of the peak amplitude, respectively, using the same expression system (HEK-293), whereas our value of persistent current was 0.76% at 23°C. Late \( I_{\text{Na}} \), however, slowly decayed in a time-dependent manner, a finding not previously recognized. The magnitude of late \( I_{\text{Na}} \), therefore, will depend on the time of measurement. We estimated the ratio of persistent current at 240 ms, whereas previous reports used earlier time points. Therefore, the observed ratio is likely higher than previously reported.

Because of the potential importance for late currents in the pathogenesis of the LQT syndrome, we also closely examined the temperature dependence of late currents. Perhaps surprisingly, no significant difference in late \( I_{\text{Na}} \) normalized to peak \( I_{\text{Na}} \) was noted for 23°C versus 33°C, and a significant difference in late \( I_{\text{Na}} \) between the wild-type current and ΔKPQ was maintained at each temperature. With more rapid decay at higher temperature, late current might be expected to be smaller at higher temperature. The third or slowest time constant for ΔKPQ \( I_{\text{Na}} \) decay, however, was not significantly decreased at higher temperatures in contrast to the first and the second time constant (Fig. 3, C–F). Slower recovery of late \( I_{\text{Na}} \) compared with peak \( I_{\text{Na}} \) was found at both temperatures (Fig. 6). These results suggest that the late \( I_{\text{Na}} \) kinetics (slow inactivation decay and slow recovery) are different from peak \( I_{\text{Na}} \) and also correspond with the reported mechanisms that the late \( I_{\text{Na}} \) in the ΔKPQ may result from mode switching to the slow gating kinetics (3, 4, 6).
Fig. 6. Time course of recovery from inactivation at 23 and 33°C. Membrane potential was stepped to −20 mV from a holding potential of −120 mV for 1 s, and a test pulse to −20 mV was delivered followed by a variable recovery interval (ΔT) to −120 mV, as indicated in inset. Currents were normalized to the maximum current recorded in the absence of a conditioning pulse and plotted against the recovery time on a logarithmic axis. Time course of recovery for peak I_{Na} (WT: dotted lines; ΔKPQ: solid lines) at both temperatures was best fit with a double exponential function: normalized I_{Na} = 1 - (A_1 × exp −t/τ_1 + A_2 × exp −t/τ_2), where τ is the recovery time. Time course of recovery for late I_{Na} in ΔKPQ measured at 240 ms (ΔKPQ240ms) was also plotted to compare with peak I_{Na}. Note that late I_{Na} did not deactivate completely in the prepulse; thus the recovery process begins from -0.42 normalized I_{Na}. Mean data for recovery of late I_{Na} was fitted with two components with offset 1 - (A_1 × exp−t/τ_1 + A_2 × exp−t/τ_2 + offset) at 23°C and a single component with offset 1 - (A_1 × exp−t/τ_1 + offset). Values of parameters are summarized in Table 2.

lower value for late current that we report results, in part, from the time-dependent decay of late I_{Na}. Late I_{Na} measured in our preparation at the same time points as those reported previously, however, still yielded a smaller relative late I_{Na} amplitude.

Although no significant difference in midpoints of steady-state inactivation between the wild type and ΔKPQ were detected in the present study, the slope factors at 23°C were significantly different. Depending on the extent to which macroscopic current decay represents inactivation, this could be consistent with the observed decrease in the voltage dependence of decay rates for ΔKPQ (Fig. 3C). Macroscopic decay, however, is a convolution of the kinetics of activation and inactivation, and the changes in steepness of the inactivation curve and the decay curves may also indicate an alteration of coupling between the activation and inactivation process.

Implication for the clinical phenotype. The rapid onset of fast inactivation and faster recovery from inactivation for the ΔKPQ are generally reported findings (1, 22). Recently, fast recovery from inactivation was reported as a possible mechanism for arrhythmogenesis in the SCN5A mutation that caused idiopathic ventricular fibrillation (5). Although the persistent current of I_{Na} contributing to prolongation of the Q-T interval is likely to be an important mechanism for arrhythmogenesis, the complex gating changes in the present study suggest that the arrhythmogenic effects may not be limited to Q-T prolongation in the ΔKPQ but may also be exacerbated by altered excitability of the channel and effects on conduction and refactoriness because of these altered kinetics.

A new finding in this paper was that late I_{Na} recovered from inactivation more slowly than peak I_{Na}. The time constants are such that late I_{Na} would be decreased with high-frequency depolarization because of accumulation of inactivation. This mechanism may explain the shortening of the prolonged Q-T interval with increased heart rate reported in the LQT3 patients (20) and the shortening of action potential duration by rapid pacing in the experimental model for LQT3 (19).

Table 2. Parameters of recovery at different temperatures

<table>
<thead>
<tr>
<th></th>
<th>23°C</th>
<th></th>
<th>33°C</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>ΔKPQ</td>
<td>ΔKPQ240ms</td>
<td>WT</td>
</tr>
<tr>
<td>τ_1, ms</td>
<td>6.3 ± 0.7</td>
<td>4.4 ± 0.5*</td>
<td>13.6 ± 6.3</td>
<td>3.3 ± 0.6†</td>
</tr>
<tr>
<td>τ_2, ms</td>
<td>143 ± 13</td>
<td>129 ± 16</td>
<td>1.028 ± 216</td>
<td>65.2 ± 8.4†</td>
</tr>
<tr>
<td>A_0</td>
<td>0.16 ± 0.02</td>
<td>0.18 ± 0.01</td>
<td>0.44 ± 0.03</td>
<td>0.52 ± 0.04</td>
</tr>
<tr>
<td>Offset</td>
<td>0.42 ± 0.02</td>
<td></td>
<td>0.42 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, no. of experiments. ΔKPQ240ms, ΔKPQ measured at 240 ms; τ_1, fast time constant; τ_2, slow time constant; A_0, fractional amplitude of slow component. Single component fit for ΔKPQ240ms at 33°C is reported where the F-ratio did not support fitting with two components (see MATERIALS AND METHODS), and the time constant for the single component fit was arbitrarily assigned to the τ_2 row.

*Significant difference compared with WT at P < 0.05. †Significant difference compared with 23°C at P < 0.01.
Human heart \( I_{Na} \) demonstrates more rapid gating kinetics with increased temperature similar in magnitude to that in previous reports in nonhuman channels. A mutant channel, \( \Delta KPQ \), associated with the congenital LQT exhibits complex gating changes, including a relative increase in late currents that may account for the clinical phenotype. This late current shows a gradual decay and slow recovery from inactivation, indicating that it is subject to a slow inactivation process.

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