Modulation of $I_{Kr}$ inactivation by mutation N588K in KCNH2: A link to arrhythmogenesis in short QT syndrome

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Abstract

Objective: Short QT syndrome (SQTS) is characterized by ventricular arrhythmias and sudden death. One form of SQTS is caused by mutation N588K in human ether-a-go-go-related gene (HERG). In this study we sought to determine the potential role of N588K in arrhythmias.

Methods: We measured the characteristics of HERG current generated by wild-type (WT) KCNH2 and the N588K mutant channel expressed in mammalian TSA201 cells.

Results: Whole-cell patch-clamp recordings of WT HERG currents showed the usual rapid onset of inactivation (rectification) at potentials more positive than +10 mV. In contrast, N588K currents rectified at potentials over +80 mV. Over the physiological range of potentials, N588K currents do not inactivate. During an action potential clamp, WT currents displayed a "hump" like waveform with slow activation kinetics and a rapid increase during phase 3 repolarization. In contrast, N588K currents were proportional to the amplitude of the action potential and displayed a dome-like configuration and a much larger current during the initial phases in the ventricle. Purkinje cell action potentials display a more negative phase 2 repolarization than the ventricle and elicited much smaller WT and N588K currents of similar amplitudes.

Conclusions: Physiologically the N588K mutation abolishes rectification of HERG currents and specifically increases $I_{Kr}$ in the ventricle with minimal effects on the Purkinje fiber action potential duration. Such preferential prolongation may explain the separation of the T and U waves observed in the ECG of SQTS patients and lead to re-excitation of the ventricle endocardium.

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1. Introduction

The duration of the QT interval on the ECG is primarily determined by the duration of the ventricular action potentials (APD). The amplitude of the rapid ($I_{Kr}$) and slow ($I_{Ks}$) components of the delayed rectifier potassium currents are major determinants of the repolarizing forces modulating APD. The short QT syndrome (SQTS) is a clinical entity recently described [1] and characterized by a short QT interval in the ECG, episodes of paroxysmal atrial fibrillation and sudden cardiac death (SCD) in patients with structurally normal hearts. To date SQTS has been identified in 12 families mainly in Europe. Our group initially linked an inherited form of SQTS (SQTS1) to a gain of function in $I_{Kr}$ [2]. A second inherited SQTS form (SQTS2) was subsequently linked to a gain of function in $I_{Ks}$ [3].
The protein responsible for $I_{Kr}$ is formed by a tetramer composed of homologous KCNH2 core units. Co-assembly of KCNH2 with the MiRP1 subunit (KCNE2) is required to fully reproduce the biophysical and pharmacological properties of the native $I_{Kr}$ [4]. SQT1 was linked to separate missense mutations identified in three families with a high incidence of SCD and SQTS, each resulting in the replacement of an asparagine by a lysine at position 588 (N588K) in KCNH2 [2]. We previously showed that N588K substitution induced a remarkable gain of function in human ether-a-go-go-related gene (HERG) current by abolishing inactivation in a physiological range of membrane potentials [2]. That first study however did not provide mechanistic insights to explain the loss of inactivation or potential arrhythmogenic mechanisms associated to N588K.

Fast onset of inactivation of KCNH2 channels causes rectification of $I_{Kr}$ at depolarized potentials [5]. $I_{Kr}$ current also displays a rapid recovery from inactivation followed by slower deactivation kinetics upon repolarization. Because of these unique properties, the contribution of $I_{Kr}$ to the early plateau phases (1 and 2) of the ventricular action potential remains minimal but gradually increases with repolarization to peak near the transition between phase 2 and phase 3 of the action potential [6]. The biophysical properties of N588K channels are quite different from wild-type KCNH2 such that $I_{Kr}$ contribution to the AP is drastically increased in SQT1. While the results adequately explain the shortening of the APD in SQT1, the pro-arrhythmogenic mechanism of the mutation is not as clear. In sharp contrast to inherited or acquired forms of long QT syndrome and to the arrhythmogenic potential of many Class III antiarrhythmic agents, shortening of the APD is generally viewed as beneficial. It is therefore difficult to associate SQTS to the usual triggers and the mechanism leading to arrhythmias in SQT1 remains largely unknown.

In this study, we sought to better understand the effects of N588K on inactivation and determine mechanisms potentially leading to arrhythmias in SQT1. We report that N588K channel inactivation begins to occur at +70 mV, well outside the physiological range of membrane potentials compared to WT-KCNH2 channels. The result is repolarizing current that monotonically follows the AP waveform and differentially increases the amplitude of $I_{Kr}$ during epicardial and endocardial ventricular APs thus generating non-homogenous APD shortening within the cardiac ventricles and increasing dispersion of repolarization, a known arrhythmogenic substrate. Moreover, N588K current elicited by AP stimulus are larger with the ventricular waveform when compared to Purkinje cells. This preferential increase in $I_{Kr}$ provides us with a possible mechanism to explain the appearance and separation of the U wave in the ECG of SQTS patients. Results of this study have been presented in preliminary form [7].

2. Methods

2.1. Cell transfection/mutagenesis

KCNH2 and KCNE2 were a kind gift from Drs. A.M. Brown and S.A. Goldstein respectively. Both gene constructs were cloned from their original vector into pcDNA3.1 (Invitrogen Carlsbad, CA). N588K mutation was constructed with the GeneTailor™ site-directed mutagenesis system (Invitrogen Corp) on plasmid pcDNA3.1 containing KCNH2 and the appropriate primers as previously described [2]. The mutated plasmid was sequenced to ensure the presence of N588K mutation without spurious substitutions. For transfection, KCNH2 and KCNE2 cDNA were kept at a constant molar ratio of 1:20. Modified human embryonic kidney cells (TSA201) were co-transfected with the same amounts of pcDNA-KCNH2/KCNE2 and pcDNA-N588K/KCNE2 complex using the calcium phosphate precipitation method. Cells were grown on polylysine coated 35-mm culture dishes and placed in a temperature-controlled chamber for electrophysiological study (Medical Systems, Greenvale, NY) 2-day post-transfection.

2.2. Electrophysiology

Voltage clamp recordings from transfected TSA201 cells were made using patch pipettes fabricated from borosilicate glass capillaries (1.5 mm O.D., Fisher Scientific, Pittsburg, PA). The pipettes were pulled using a gravity puller (Narashige Corp.), coated with Sylgard and filled with pipette solution of the following composition (mmol/L): 10 KCl, 125 K-aspartate, 1.0 MgCl2, 10 HEPES, 10 NaCl, 5 MgATP and 10 EGTA, pH 7.2 (KOH). The pipette resistance ranged from 1 to 4 MΩ when filled with the internal solution. The perfusion solution contained (mmol/L): 130 NaCl, 5 KCl, 1.8 CaCl2, 1. MgCl2, 2.8 Na acetate, 10 HEPES, pH 7.3 with NaOH. Current signals were recorded using a MultiClamp 700A amplifier (Axon Instruments Inc., Foster City, CA) and series resistance errors were reduced by about 60–70% with electronic compensation. All recordings were made at room temperature.  

2.3. Measurement of action potentials

Single Purkinje and ventricular myocytes were isolated from hearts using techniques previously described [8,9]. Action potentials of single cells were recorded using whole-cell patch pipettes coupled to a MultiClamp 700A amplifier. The resistance of the electrodes was 2–4 MΩ when filled with the pipette solution (described above). Action potentials were elicited using a 3-ms current pulse at 120% threshold amplitude. Both the ventricular and Purkinje cells were paced at a cycle length of 0.5 Hz. The pre-recorded action potentials served as the waveforms for the AP clamp experiments. The investigation conforms to the Guide for
the Care and Use of Laboratory Animals published by the US NIH.

2.4. Data acquisition and analysis

All signals were acquired at 10–50 kHz (Digidata 1322, Axon Instruments) with a microcomputer running Clampex 9 software (Axon Instruments, Foster City, CA). Membrane currents were analyzed with Clampfit 9 software (Axon Instruments, Foster City, CA). Results from pooled data are presented as Mean ± S.E.M. and n represents the number of cells in each experiment. A Student’s t-test was used for comparing paired data and a p<0.05 value was considered statistically significant.

3. Results

Mutation N588K is located in the S5-P linker of HERG [2], a region known to influence inactivation of the channel [10,11]. To determine if mutation N588K could also affect steady state activation, we first compared the wild-type (WT) and N588K currents elicited by 800-ms depolarizing pulses in transfected TSA201 cells. WT currents activated rapidly during step depolarizations to positive potentials and displayed the characteristic tail current generated by channels recovering upon repolarization (Fig. 1A). The amplitude of the active current at the end of the depolarizing pulse (steady state) saturated at 0 mV (Fig. 1C) and decreased at more positive potentials because of the faster onset of inactivation [5]. In contrast, N588K steady state currents increased monotonically over the range of membrane potentials tested (Fig. 1B,C). The current–voltage relationship (Fig. 1C) shows that mutation N588K shifted the threshold for activation from −50 mV in WT to −30 mV in N588K and abolished the strong rectification of the WT current at potentials over 0 mV.

WT tail currents, indicative of the maximum amount of channels activated during the preceding pulse, reached a plateau at voltages over 25 mV (Fig. 1D). In contrast, N588K tail currents did not saturate indicating that pulses to +50 mV were insufficient to activate the all channels.

![Fig. 1. Mutation N588K removed rapid inactivation of the current in a physiological range of membrane potentials. (A) Wild-type KCNH2/KCNE2 currents elicited by 800-ms depolarizing pulses in TSA201 transfected cells. Activation currents were elicited by depolarizing pulses in +10-mV increments. A large inward tail current is observed upon repolarization to −100 mV. (B) Same protocol as in A applied to N588K. Tail currents were reduced by the mutation. (C) Current–voltage relationship for steady state current measured at the end of the activating pulse. Current amplitude was largest in N588K despite transfection with equivalent molar ratios. (D) Tail current–voltage relationship as a function of the activating step potential. WT: n=8, N588K: n=11.](image-url)
Fig. 2. Inward rectification observed for wild-type (WT) HERG currents is not observed in N588K. (A) Representative traces of WT HERG and N588K fully activated currents. (B) Peak tail current amplitude was plotted as a function of the test potential. The N588K $I-V$ relationship was linear in a physiological range of potentials. In addition, N588K reversal potential was positively shifted suggesting a change in K$^+$/selectivity. WT: $n=9$, N588K: $n=11$.

Fig. 3. Paradoxical modulation of HERG current by external potassium is abolished by mutation N588K. (A) Comparison of membrane currents recorded from a N588K cell exposed to 2, 5, and 10 mmol/L external K$. The cell was held at $-80$ mV and membrane currents were elicited by stepping the voltage to membrane potentials between $-50$ and $+60$ mV. Changing external K$^+$ had little effect on the magnitude of the activating currents but the tail current amplitude linearly increased with increasing K$^+$ concentrations. (B) Mean $I-V$ relation showing that developing current was unaffected by changes in external K$^+$. (C) Peak tail current amplitude as a function of the preceding pulse amplitude from recordings as presented in A.
available. Previous studies showed that onset of inactivation is responsible for rectification of the WT current and that channels quickly recovering from inactivation generate the large tail currents observed upon repolarization. Since we did not observe saturation of the tail current, our results therefore suggest that N588K channels did not inactivate significantly in a physiological range of membrane potentials when compared to WT. To confirm this hypothesis, we looked at the rectification properties of N588K and WT channels by activating channels at +40 mV and measuring the instantaneous current upon return to membrane potentials between −120 and −40 mV (full I–V relationship, Fig. 2). The peak tail current during the recovering pulse is proportional to the maximum number of channels available at each voltage. WT peak tail currents exhibited strong inward rectification characterized by a region of negative slope conductance for membrane potentials above −40 mV (Fig. 2A,B), as previously described [5,12]. In contrast, the full I–V relation of N588K was essentially linear thus confirming that most channels were already activated at +40 mV and little inactivation occurred over the range of voltages tested. Furthermore, the reversal potential was shifted by +8 mV suggesting that mutation N588K altered K⁺ permeability. It is now well established that HERG channels display a paradoxical sensitivity to extracellular potassium such that its current amplitude increases as the external K⁺ concentration rise up to 10 mmol/L, despite the corresponding reduction in driving force [12–14]. We next tested if the N588K current exhibited the same anomalous dependence on external K⁺. Surprisingly, changing external K⁺ concentration from 2 to 5 and 10 mmol/L (hence reducing the driving force of the current) had little effect on the magnitude of N588K activating (steady state) outward current (Fig. 3A,B) while the amplitude of the tail current, an indication of the maximal number of activated channels, increased proportionally with increasing external K⁺ concentrations (Fig. 3C).

The lack of increase in N588K active current and the increase in tail current as extracellular potassium concentration ([K₀]) was elevated suggest that the increase in K⁺ driving force may have partially restored C-type inactivation. Alternatively, the selective reduction of the outwardly going current may be due to altered K⁺ permeability. We tested each hypothesis by comparing the full I–V relationship of N588K and WT under different concentrations of external potassium.

Fig. 4A shows that WT currents still rectify, albeit at more positive potentials as the concentration of external potassium was raised. The same increase in external potassium did not induce rectification of N588K current (Fig. 4B). The current voltage relationship deviated from linearity as the potassium concentration was raised such that as to yield a cross over of the outward currents at +30 mV, suggesting that mutation N588K altered the selectivity of the channel. Measured reversal potentials were significantly higher (p<0.05) for WT (n=7) vs. N588K (n=7) with values of −85 ± 2 mV, −65 ± 2 mV, −50 ± 2 mV (WT) and −73 ± 2 mV, −52 ± 3 mV, −32 ± 6 mV (N588K) in external potassium concentrations of 2, 5 and 10 mmol/L, respectively. Evaluation of the relative permeability (P_K/P_Na) using the simple bionic selectivity equation $E_r = \frac{58.2}{\log \left( \frac{E_K}{E_N} \right)}$, where $E_r$ stands for reversal potential and $E_K$ and $E_N$ represent the intracellular and extracellular concentrations of Na⁺ and K⁺ yielded permeability ratio values of 0.062 ± 0.004 and 0.110 ± 0.004 for WT and N588K, respectively (p<0.01), thus showing that the channel selectivity was reduced.

Our results thus far indicated that N588K currents did not inactivate over the physiological range of potentials tested. We next wondered if N588K channels failed to rectify completely or rectified at more positive potentials because of the positive charge introduced in the pore of the channel by the lysine. To test this hypothesis we measured N588K currents at activation voltages as high as +150 mV. Fig. 5A shows representative current recordings elicited by step potentials as high as +110 mV. N588K steady state current amplitude peaked at +80 mV and decreased at more positive potentials.
potentials. Rectification typically occurred abruptly between +70 to +100 mV. In two cells, however, rectification occurred above +130 mV and in another one it failed to occur. Fig. 5B shows such current recordings in which rectification occurred abruptly at +100 mV with a ~4-nA reduction in amplitude. As opposed to WT channels, however, this strong rectification did not result in an increase in tail current upon repolarization to -100 mV, thus suggesting different inactivation mechanisms for WT and N588K currents.

To test if inactivation of N588K could account for the more positive maximum of N588K current–voltage relationship (Fig. 5), we assessed the voltage dependence of WT and N588K channels availability (Fig. 6). Channel availability was estimated by plotting the normalized tail current amplitude (to peak) following the conditioning potential against the voltage of their respective recovery pulse (Fig. 6C). A Boltzmann distribution function fitted to the normalized amplitudes yielded mid-inactivation potentials of -48.2±0.6 mV and +54.1±1.4 mV for WT and N588K, respectively. Thus, N588K channels inactivate at voltages more positive than the physiological range of membrane potentials. We next sought to determine how the shift in channels availability introduced by N588K could generate an arrhythmogenic substrate.

Since WT channels inactivate during the plateau of the ventricular AP and recover rapidly at the onset of the repolarization, \( I_{Kr} \) reaches its maximum amplitude during phase 3 repolarization and contributes minimally to the early phases of the AP [2] (Fig. 7A). Because of the lack of inactivation, we hypothesized that N588K current will contribute more than WT to the early phases of the ventricular AP and may prematurely trigger repolarization.

To test this hypothesis, we compared the kinetics of WT and N588K currents elicited from previously recorded canine epicardial (Epi) or endocardial (Endo) AP waveform (Fig. 7A). In response to either the Epi- or Endo-AP waveform (Fig. 7A), WT currents displayed a typical hump-like waveform with slow activation kinetics and a rapid increase during the phase 3 repolarization, due to the
rapid recovery of inactivated channels (Fig. 7A, middle panel). The maximum amplitude of the WT current during phase 3 was not altered by the amplitude of phase one (notch) of the Epi action potential. In sharp contrast, N588K currents monotonically followed the amplitude of the AP waveform and displayed a domelike configuration resulting in a much larger current during the initial phases (1 and 2) of the action potential. Moreover, the presence of a deep notch (phase 1) in the epicardial AP waveform significantly reduced the maximal amplitude of the current (Fig. 7A, bottom panel). Peak N588K currents activated using an epicardial AP waveform were 42 ± 7% smaller than N588K currents activated by the endocardial AP waveform (n = 5). These results show that as opposed to WT, the current generated by N588K is strongly modulated by the amplitude of phase 1 repolarization. This may have significant implications for the APD, modulation of the refractory period and the conduction/propagation in the ventricle.

Purkinje fibers normally display a longer APD and lower plateau amplitude than ventricular APs [8,15]. Early studies linked the longer APD of Purkinje fibers to the occurrence of a secondary wave immediately following and sometimes partially overlapping the T wave and termed the U wave [16,17]. Such U waves were also observed in SQTS patients [18]. Since our results indicate that N588K mutation confers to \( I_{Kr} \) a monotonic response to AP depolarization, we wondered if differences in the amplitude of the AP plateau could influence the amplitude of WT and N588K currents and provide some insight into the generation of the U wave in SQTS. Because of the lower plateau of the Purkinje cell AP, we hypothesized that mutation N588K should significantly reduce the amplitude of N588K current during a Purkinje cell action potential. Fig. 7B shows that in response to a Purkinje cell AP, N588K showed a much smaller current amplitude compared to the ventricular AP waveform thus suggesting that the N588K-induced shortening of the AP will be more important in the ventricles than in Purkinje fibers.

Since N588K currents responded differently than WT to changes in extracellular potassium, we next looked at the response of N588K channels to a ventricular action potential stimulus in different [K\text{o}]s. Fig. 8C shows that 2 mmol/L, 5 mmol/L and 10 mmol/L [K\text{o}] had little effect on the maximal amplitude of N588K during an action potential, as anticipated from the results presented in Fig. 3.
4. Discussion

4.1. Summary of main findings

Our results indicate that mutation N588K shifted inactivation of HERG towards more positive potentials causing a functional increase in $I_{Kr}$. As a result N588K currents increase proportionally to the level of depolarization at physiological membrane potentials while WT currents decrease (rectify) at potentials more positive than $-10$ mV. These findings led us to consider that WT and N588K may react differently during the course of an AP.

In AP clamp experiments, we found that N588K channels monotonically followed the shape and amplitude of the action potential used as the stimulus such that a more depolarized phase 1 repolarization yielded larger current amplitudes. In contrast, WT channels rapidly inactivated and yielded similar current amplitudes during phase 1 repolarization of the Epi and Endo action potentials but recovered rapidly during repolarization to generate a large current during phase 3.

Fig. 7. The amplitude of N588K currents is more dependent on the early phase of the action potential than WT. (A) Pre-recorded action potentials from the ventricular epicardium and endocardium of dog hearts were used as stimulus to elicit wild-type (WT) (middle panel) and N588K (bottom panel) currents. N588K currents monotonically followed the amplitude of the ventricular action potentials and reached larger amplitudes than WT during both stimuli. The more depolarized phase 1 of the endocardial action potential significantly increased the maximal amplitude of N588K current but had little effect on WT. (B) Representative currents elicited during an action potential clamp using pre-recorded APs from a ventricular and Purkinje cell. N588K maximal current amplitude elicited by the Purkinje cell waveform was much smaller than the response generated by the ventricular action potential.

Fig. 8. The contribution of N588K current to the ventricular action potential is minimally modulated by physiological changes in external potassium. Current recordings as presented in Fig. 7A from N588K channels exposed to 2, 5 and 10 mmol/L extracellular potassium show that the maximum amplitude of the current remains unchanged.
Purkinje cells have a lower phase 2 voltage, N588K current remains small throughout the APD in Purkinje cells but increases drastically by the more positive plateau of the ventricular AP.

### 4.2. N588K biophysical changes

Our results demonstrate that N588K channels rectify at potentials greater than +70 mV (more positive than the physiological range of potentials). The result of this shift of rectification is that the magnitude of current is much greater at more positive potentials. Interestingly, when N588K channels did inactivate at potentials greater than +70 mV, no dramatic increase in the magnitude of the tail current was observed upon repolarization. These observations as well as the abrupt rectification of the current (compared to WT) may suggest the presence of a blocking particle interacting with N588K. Alternatively, the introduction of positive charge (lysine) may alter the S5-P segment configuration (Fig. 9). Since inactivation of the current occurs at more positive potentials, we speculate that the α-helical structure of the S5-P segment is preserved but that the energy required for the conformational change leading to C-type inactivation is increased by the addition of a positive charge. The gating changes caused by the introduction of the lysine at the mouth of the channel could suggest that the S5-P segment itself may act as a voltage sensor for C-type inactivation. Interestingly, a different mutation at the same amino acid position (N588D) in HERG introduces a negatively charged amino acid (aspartate) and yields the Long QT phenotype [19].

When we transfected N588D in TSA201 cells, minimal current was expressed consistent with the Long QT phenotype. Because of the minimal current expression of N588D, a detailed biophysical analysis was not performed. Further experiments will be required to determine if the effect is due to the positive charge introduced by the N588K substitution at the mouth of the channel pore or to conformational changes in the outer mouth of HERG channels.

The outer mouth of HERG channels, namely the S5-P region has been shown to be involved in C-type inactivation [10,20]. Liu et al. [11] proposed that the amino acids at positions 583–597 in the S5-P linker form an α-helix that may serve as a bridge between the outer mouth of the channel and the voltage sensor and that the NH2 terminal region may be very close to the outer entrance (Fig. 9). Our results are consistent with the involvement of N588 in C-type inactivation. At very positive potentials, inactivation of N588K is restored and the current–voltage relationship follows a bell shape waveform similar to the one observed for WT (Fig. 5). Concurrently, N588K steady state inactivation is shifted by +102 mV vs. WT (Fig. 6).

Although the experiments performed in this study were in the presence of KCNE2, some studies have questioned the contribution of KCNE2 to $I_{Kr}$ function [21,22]. In a previous study, we repeated the expression studies with N588K and KCNH2 expressed without KCNE2 [2]. We found that the mutation had similar effects on currents expressed by KCNH2 and N588K alone (Fig. 3B and D), although the augmentation of the developing current and the diminution of the tail current were not as pronounced as in the presence of KCNE2 [2].

It is well established that WT HERG currents display an anomalous decrease in current when extracellular potassium is lowered [12,14]. Decreasing extracellular potassium from 5 to 2 mmol/L decreases the amplitude of HERG maximal current by up to 40% [23] and it has been proposed that such current reduction may play a role in the prolongation of QT interval observed during hypokalemia and could explain some of the beneficial effects of a modest serum K$_e$ elevation. We wondered if the arrhythmias in patients exhibiting SQTS may be due to greater sensitivity of N588K to a reduction in extracellular K$^+$. Surprisingly, changing extracellular potassium had little effect on the maximal amplitude of N588K. This suggests that the change in driving force produced by changes in external K$^+$ is opposed by a change in conductance through the channel. In our study, analysis of the fully activated $I-V$ showed that the reversal potential for N588K was slight shifted compared to WT suggesting that there was an alteration in K$^+$ permeability.

A puzzling observation was that the amplitude of N588K active current remained the same in extracellular K$^+$ concentrations of 2, 5, and 10 mmol/L (Fig. 4B) while the inward tail currents were proportional to the K$^+$ gradient (Fig. 3D). Moreover, N588K recordings using the full I/V protocol (Fig. 4) did not indicate any current rectification for potentials up to +50 mV and showed that outward currents converged at positive potentials. These results suggest that the positively charged lysine introduced by the mutation interfered with the permeability and the selectivity of the

![Fig. 9. The mutation resulted in an amino acid change from asparagine (N) to lysine (K) at codon 588 (N588K) located in the extracellular loop between segments S5 and S6 of the Human Ether-a-go-go-Related Gene (HERG).](image-url)
channel, possibly through electrostatic or steric hindrance. Such hypothesis remains to be verified but is consistent with the role of amino acids 583 to 597 in HERG selectivity and the proposed α-helical structure of the S5-P segment [11,24].

4.3. Kinetics of N588K during an action potential

As a consequence of the lack of inactivation in the voltage range of an action potential, N588K current amplitude remains proportional to the voltage during phase 1 and phase 2 repolarization. The presence of a deep notch (phase 1) in the epicardial AP thus significantly delays the activation of N588K resulting in a smaller contribution of $I_{Kr}$ to phase 2 in epicardium when compared to endocardium (Fig. 7). The combined effects of removal of inactivation and our observation that N588K channels activated at more positive potentials than WT (Fig. 1D) are consistent with this finding. Thus, inactivation of WT channels equalizes the contribution of $I_{Kr}$ to the early phases of the Epi and Endo APs whereas the lack of inactivation in N588K channels confers to $I_{Kr}$ a higher sensitivity to the amplitude of phase 1 repolarization. Physiologically, such a differential response of $I_{Kr}$ between the epicardial and endocardial layers of the ventricle is likely to promote dispersion of repolarization, a known arrhythmogenic substrate [25,26], and could help explain the occurrence of VT/VF in SQTS.

The effects of the mutation on the inactivation of HERG can also help us to understand the appearance and separation of the T and U wave in SQTS. Purkinje cell APs are longer and characteristically display a lower phase 2 voltage than ventricular APs [8,15]. Under normal conditions, the U wave is usually buried within the T wave of the ECG, likely because of the small conduction delay between activation of Purkinje fiber and the ventricles and the difference in repolarization time between the two cell types. However, in patients with SQTS a clear separation of the T and U wave is often observed [18]. Our result shows that because of the lower phase 2 voltage, N588K current remains small throughout the APD in Purkinje cells but is increased drastically by the more positive plateau of the ventricular AP. In-vivo, ventricular cells are therefore likely to repolarize more rapidly than Purkinje cells thus accentuating the difference in APD between Purkinje cells and ventricle. This may help to explain the separation of the U wave from the T wave observed on the ECG in SQTS patients but more importantly, the APD difference between Purkinje cells and ventricle will selectively shorten the refractory period of the ventricle. Without a parallel decrease of the APD of Purkinje cells, the shorter refractory period of the ventricle is likely to increase the probability of premature ventricular re-excitation.

In conclusion, our study shows that mutation N588K induced a large positive voltage shift in the inactivation of HERG channels. As a consequence, $I_{Kr}$ currents electronically follow the waveform and amplitude of the cardiac action potential. This will result in larger amplitude $I_{Kr}$ within the ventricle when compared to the Purkinje fibers and, correspondingly, shorter ventricular APs. The net effect is likely to selectively reduce the ventricular refractory period and increase excitability such that smaller transmural dispersion of repolarization within the ventricle combined with relatively longer Purkinje cell action potential may create an arrhythmogenic substrate for SQTS-related arrhythmias.

4.4. Limitations of the study

The results from the AP clamp experiments suggest that the mutation N588K produces a dramatic increase in repolarizing current compared to WT during an action potential. This would likely result in an abbreviation of the cardiac action potential. However, caution should be exercised when making the translation to the clinical situation. Although we demonstrate that N588K produces an increase in $I_{Kr}$ current, it is unclear to what extent the cardiac action potential duration would be affected due to the presence of other currents. In a recent study, Zhang and Hancox modeled the effect of removing inactivation of $I_{Kr}$ on the cardiac action potential and QT interval [27]. They found that removal of $I_{Kr}$ inactivation resulted in a shortening of the QT interval and diminished the dispersion of repolarization. At the cellular level, loss of inactivation of $I_{Kr}$ produced a shortening of the ventricular action potential duration and this effect was greatest when $I_{Ks}$ density was smallest. These results suggest that cells in the midmyocardial region would exhibit the greatest change due to the low density of $I_{Ks}$ [28,29].

Although the present study was performed at room temperature, it is known that there are changes in HERG channel function associated with increasing the temperature to 37 °C [30]. These temperature-dependent effects on HERG may result in different channel kinetics which would alter the functional contribution of $I_{Kr}$ during the course of an action potential. In both ventricular myocytes [6] and mathematical models [27,31], it was determined that the contribution of $I_{Kr}$ was greatest during phase 3 repolarization. Consistent with these studies, our AP clamp experiments showed that the contribution of normal $I_{Kr}$ was greatest during phase 3 repolarization.

Despite a significant negative shift in steady state activation, the study of Wang and al. [30] did not indicate that the changes in $I_{Kr}$ kinetics would be different in Purkinje fibers vs. ventricular cells. Since mutation N588K abolishes inactivation in the voltage range of an action potential but has little effect on the activation kinetics, a similar shift in steady state activation by temperature will modulate the amplitude of the current monotonically rather than changing N588K current kinetics and the differences reported in our study will remain and perhaps be amplified.
Since there are no studies showing major differences in normal $I_{Kr}$ kinetics between Purkinje fibers and ventricles, it is likely that temperature will therefore modulate the amplitude of the differences we are reporting rather than changing the actual gating of the channel during an AP per se. Given that a negative shift in activation of WT channels is likely to increase the amplitude of the current more at positive potentials ($-10$ to $+20$ mV) rather than at $-50$ mV, as an example, one can intuitively argue that because of the more positive action potential plateau of the ventricular AP, the contribution of $I_{Kr}$ will be larger in Purkinje fibers which display a much more negative AP phase 2 repolarization. Thus, one can speculate based on the study of Wang et al. [30] and our results on N588K that at physiological temperatures, the difference in the contribution of $I_{Kr}$ may be larger in ventricle vs. Purkinje fibers. Our results, therefore, may underestimate the effects of the mutated channels on the amplitude of $I_{Kr}$ and the associated differences in action potential duration between Purkinje fibers and ventricular cells. Possible differences between the behavior of the HERG channels in situ and in vitro may exist. However, the clone we used is the human isoform of HERG, co-expressed with its ancillary sub-unit KCNE1. Therefore, it is likely that the main features described in this paper will appear in patients as well. In support of our data, one can note the close correlation from our action potential (EP) data and the clinical ECG (appearance of the U wave).

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References


