Action Potential Abnormalities due to Loss- or Gain-of-Function Mutations in *KCNJ2*

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Abstract

Andersen–Tawil syndrome type 1 (ATS1) and short QT syndrome type 3 (SQT3) are associated with loss-of-function and gain-of-function mutations in the KCNJ2 gene, respectively. This gene encodes the Kir2.1 protein, which is the most abundant member of the Kir2.x family in the Kir2.x tetramers that constitute the channels that conduct the cardiac inward rectifier potassium current (I_{K1}).

The effects of ATS1 and SQT3 related mutations in KCNJ2 on the electrophysiological characteristics of human ventricular cells were assessed in computer simulations using the updated ten Tusscher et al. human ventricular cell model. The model I_{K1} was replaced with either wild-type or heterozygous mutant Kir2.1 current.

In ATS1 simulations, the action potential was only modestly prolonged and calcium-driven spontaneous action potentials could be observed. The resting membrane potential was depolarized by 7 mV, thereby reducing sodium channel availability and thus contributing to a noticeable decrease in conduction velocity. In SQT3 simulations, effects on resting membrane potential and conduction velocity were relatively small. However, action potentials with a markedly shortened duration, increasing the susceptibility to tachyarrhythmias, could be elicited.

1. Introduction

Andersen–Tawil syndrome (ATS), named after clinical investigators Ellen Damgaard Andersen and Rabi Tawil, is a rare inheritable multisystem disorder. Ventricular arrhythmias, periodic paralysis and dysmorphic features constitute the classic triad of ATS symptoms [1,2]. In 60–70% of the ATS patients a mutation in the *KCNJ2* gene can be identified (ATS type 1) [3]. This gene encodes the Kir2.1 protein, which is the most abundant member of the Kir2.x family in the human heart. Arranged in tetramers, Kir2.1 proteins constitute the channels that conduct the cardiac inward rectifier potassium current (I_{K1}). So far, over 60 different autosomal dominant mutations in the *KCNJ2* gene have been associated with ATS [3]. Most of these loss-of-function mutations have a dominant-negative effect, which means that a single mutated Kir2.1 channel subunit can significantly reduce the current through the channel. Thus, the I_{K1} current amplitude is typically reduced to 20–30% of control or even less [4].

A multitude of cardiac symptoms is found in ATS patients, including an elongated QT interval and prominent U waves on the ECG, and several types of ventricular arrhythmias [3]. The prolongation of the QT interval explains the classification of ATS1 as long QT syndrome type 7 [4]. However, on average the QT interval is only marginally prolonged [3].

Autosomal dominant mutations in the *KCNJ2* gene have also been associated with short QT syndrome (SQT syndrome type 3). SQT3 is rare and so far only a small number of mutations has been uncovered and functionally characterized. Specifically, these are the D172N [5], M301K [6], E299V [7], and K346T mutations [8]. These gain-of-function mutations either increase the I_{K1} amplitude (D172N, K346T) or weaken its rectification (E299V, M301K).

2. Methods

The functional effects of loss- or gain-of-function mutations in *KCNJ2* were assessed by computer simulations using the human ventricular cell model by ten Tusscher et al. [9], as updated by ten Tusscher and Panfilov [10] (TNNP06 model). The model equation for I_{K1} was replaced with an equation based on the experimental data on wild-type (WT) Kir2.1 current of Dhamoon et al. [11]:

$$I_{K1} = A \times (V_m - E_K) / (1 + \exp(B \times (V_m + C))), \quad (1)$$

where the membrane potential V_m and potassium reversal potential E_K are in mV and I_{K1} is in pA/pF. The parameters A, B, and C amounted to 0.12979 (nS/pF), 0.093633 (mV^{-1}) and 72.0 (mV), respectively.

The same equation, but with different parameter settings, was used to describe I_{K1} for the heterozygous D172N and E299V SQT3 mutations, in accordance with the experimental data on WT/D172N and WT/E299V

Kir2.1 current of Deo et al. [7]. A, B, and C were set to 0.44870, 0.089952 and 71.936 for WT/D172N and 0.98765, 0.020133 and 170.26 for WT/E299V, respectively. ATS1 was simulated through a decrease of the I_{K1} amplitude to 25% of control. The associated I_{K1} current-voltage relationships are shown in Figure 1.

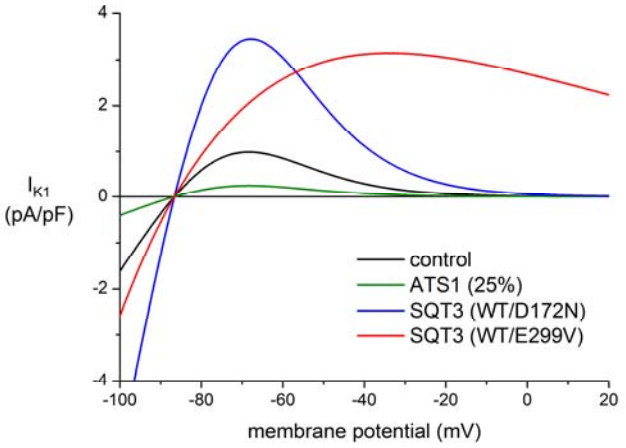

Figure 1. Current-voltage relationships of control (wild-type) and ATS1 and SQT3 mutant I_{K1}. Control outward current amplitude is 1 pA/pF (at −68 mV).

Conduction velocity was determined in a linear strand of 80 longitudinally coupled cells, in which intercellular coupling conductance was set to 10 μS and myoplasmic resistivity amounted to 150 Ω·cm. All simulations were run for a sufficiently long time to reach steady-state behavior.

3. Results

3.1. Action potential observations

First, the TNNP06 model cell was stimulated with a 1-ms, 9.2-nA stimulus current at a frequency of 1 Hz to elicit action potentials and assess changes in action potential characteristics as a result of heterozygous ATS1 and SQT3 mutations. The acquired action potentials and associated I_{K1} current traces are shown in Figure 2.

The control action potential (Figure 2A, black trace) shows a resting membrane potential (RMP) of −84 mV, a maximum upstroke velocity ($\dot{V}_{max}$) of 328 V/s and an action potential duration (APD), measured at a membrane potential level of −72 mV, of 320 ms. For ATS1, simulated by reducing I_{K1} amplitude to 25% of control, APD was only modestly prolonged by 16 ms, whereas RMP was depolarized by 7 mV (Figure 2A, green trace). This 7-mV depolarization resulted in a substantial inactivation of sodium channels, as reflected in an almost 2-fold decrease in $\dot{V}_{max}$ to 166 V/s. In contrast, each of the two SQT3 mutations causes a small hyperpolarization of RMP (1–2 mV; Figure 2A, red and blue traces) and thereby a 20–30 V/s increase in $\dot{V}_{max}$.

Both SQT3 mutations shorten the action potential. For WT/D172N, the shortening is limited to 22 ms, whereas it is as large as 230 ms for WT/E299V, with a remaining APD of only 90 ms. In the latter case, the shallow rectification (cf. Figure 1) is responsible for a substantial I_{K1} in the membrane potential range of the action potential plateau (Figure 2B), which results in almost complete suppression of this plateau (Figure 2A). In contrast, the main effect for WT/D172N is an acceleration of the final phase of repolarization.

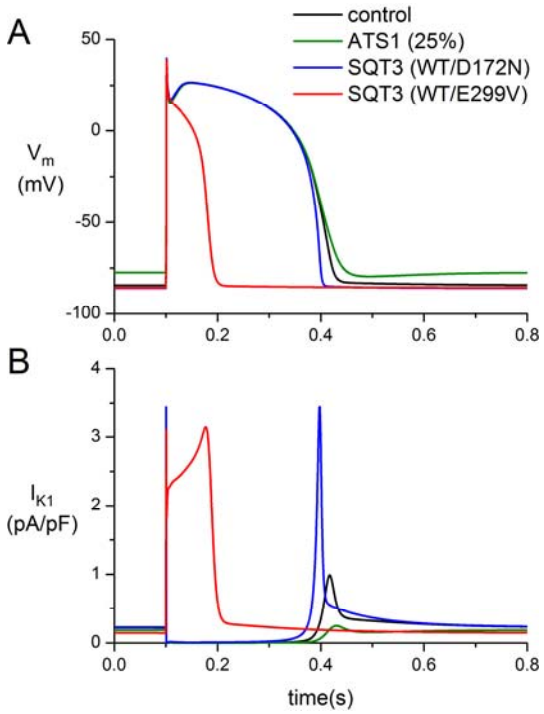

Figure 2. (A) Action potentials elicited at 1 Hz and (B) associated I_{K1}.

The findings regarding the APD are in line with the clinical observations in terms of the more severe effects of the E299V mutation compared to the D172N mutation. However, the 22-ms shortening for WT/D172N is smaller than would be expected from the rate-corrected QT interval (QTc interval) of 315 ms of both the proband and her father [5]. The dramatic shortening for WT/E299V may be somewhat larger than anticipated, even though the proband with the heterozygous E299V mutation showed a QT interval that was so short that the QRS complex merged with the T wave [7].

If, by further reducing I_{K1} amplitude to 10% of control, a more severe type of ATS1 is simulated, calcium-driven spontaneous action potentials can be observed (Figure 3A). The action potential repolarization to a maximum diastolic potential of −77 mV is followed by an almost

linear spontaneous diastolic depolarization at a rate of ≈26 mV/s. Accordingly, the amplitude of the underlying net inward current (I_{net}) amounts to ≈0.026 pA/pF (Figure 3B, top panel). It is the calcium transient that triggers an inward sodium-calcium exchange current (I_{NaCa}) with an amplitude exceeding that of I_{K1} (Figure 3B, bottom panel), thus generating pacemaker activity at a rate of 1.1 Hz.

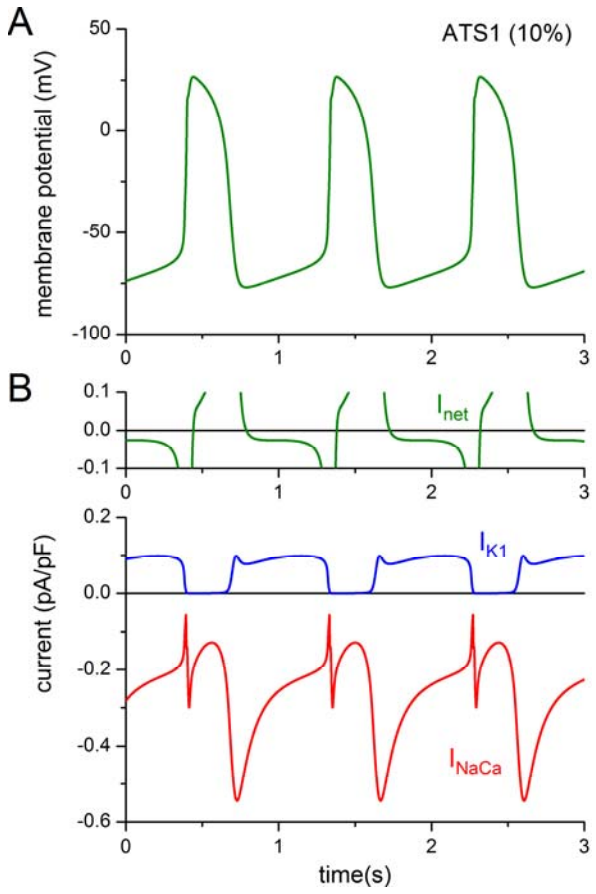

Figure 3. Pacemaker activity upon reducing I_{K1} amplitude to 10% of control, simulating a more severe type of ATS1. (A) Calcium-driven spontaneous action potentials. (B) Associated net inward current (I_{net}), sodium-calcium exchange current (I_{NaCa}), and I_{K1}.

3.2. Restitution properties

Next, an S1-S2 protocol was applied to determine cellular restitution properties. A series of 200 action potentials elicited at 1 Hz with a 1-ms, 9.2-nA S1 stimulus was followed by an S2 stimulus at various S1-S2 intervals. The duration and peak sodium current of the action potential elicited with the S2 stimulus are shown in Figure 4, A and B, respectively.

The effects of the heterozygous D172N mutation on APD restitution are relatively small (Figure 4A). This is in line with simulation results regarding the D172N mutation in previous studies, which employed other human ventricular cell models [5,7,12]. An important observation, however, is the small APD of 110–120 ms at an S1-S2 interval of 315–320 ms for WT/D172N, which increases the susceptibility to tachyarrhythmias. The flat APD restitution curve for WT/E299V (Figure 4A, red line with triangles) is in line with the clinically observed flat relationship between heart rate and QT interval [7].

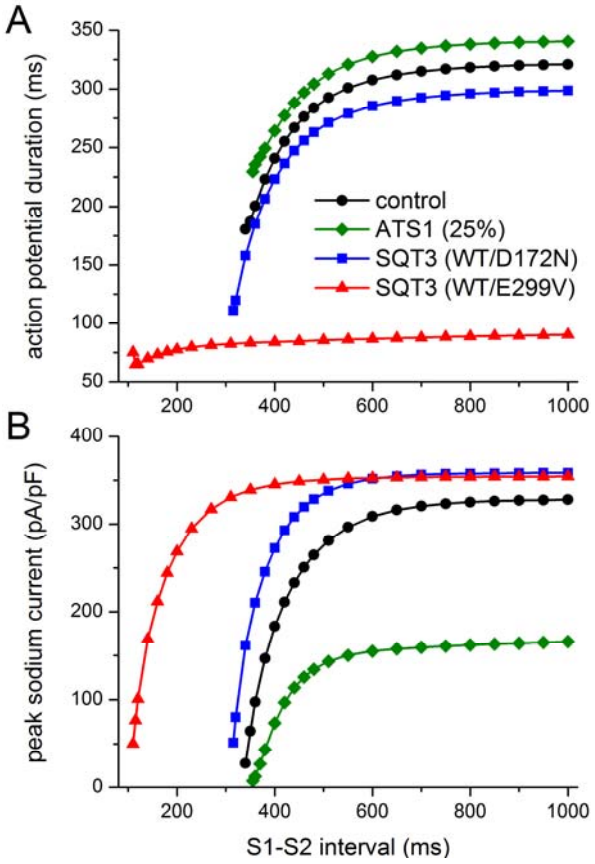

Figure 4. Cellular restitution properties as determined with an S1-S2 stimulus protocol. (A) Action potential duration. (B) Peak sodium current.

The two SQT3 mutations show an increase in peak sodium current, in particular at short-coupled intervals (Figure 4B), which further facilitates tachycardias. A remarkable decrease in peak sodium current occurs for ATS1, as could be anticipated from the aforementioned almost 2-fold decrease in $\dot{V}_{max}$ at a stimulus frequency of 1 Hz [13].

3.3. Conduction velocity

The effects of the ATS1 and SQT3 mutations on conduction velocity were assessed in simulations of a linear strand of TNNP06 cells paced at 1 Hz. For both

SQT3 mutations, the conduction velocity was highly similar to the control value of 59 cm/s (Figure 5). For ATS1, however, there is a decrease to 49 cm/s, reflecting the aforementioned reduced availability of sodium current.

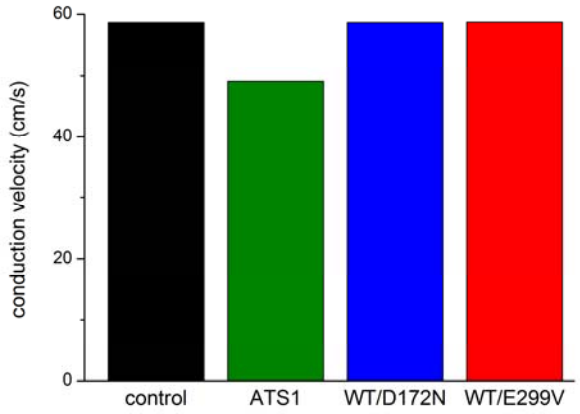

Figure 5. Conduction velocity as determined in a linear strand of 80 longitudinally coupled cells at a pacing frequency of 1 Hz.

4. Conclusion

The above simulation results show proarrhythmic action potential changes upon both loss-of-function and gain-of-function mutations in *KCNJ2*. These may explain the susceptibility to ventricular arrhythmias observed in ATS1 and SQT3 patients. The effects on the action potential are in line with the clinical ECG observations, except for the reduced conduction velocity in ATS1, which would be reflected in QRS prolongation. Such prolongation has been observed in transgenic mouse models of ATS1, but not in ATS1 patients.

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