Mechanistic Links Between Na\(^+\) Channel (SCN5A) Mutations and Impaired Cardiac Pacemaking in Sick Sinus Syndrome

Timothy D. Butters,* Oleg V. Aslanidi,* Shin Inada, Mark R. Boyett, Jules C. Hancox, Ming Lei, Henggui Zhang

Rationale: Familial sick sinus syndrome (SSS) has been linked to loss-of-function mutations of the SCN5A gene, which result in decreased inward Na\(^+\) current, \(I_{\text{Na}}\). However, the functional role of \(I_{\text{Na}}\) in cardiac pacemaking is controversial, and mechanistic links between mutations and sinus node dysfunction in SSS are unclear.

Objective: To determine mechanisms by which the SCN5A mutations impair cardiac pacemaking.

Methods and Results: Action potential (AP) models for rabbit sinoatrial node (SAN) cells were modified to incorporate experimentally reported \(I_{\text{Na}}\) changes induced by 2 groups of SCN5A gene mutations (affecting the activation and inactivation of \(I_{\text{Na}}\), respectively). The cell models were incorporated into an anatomically detailed 2D model of the intact SAN-atrium. Effects of the mutations and vagal nerve activity on cardiac pacemaking at the single-cell and tissue levels were studied. Multi-electrode extracellular potential recordings of activation patterns from intact SAN-atrium preparations were performed to test predictions of the models. At the single-cell level, the mutations slowed down pacemaking rates in peripheral, but not in central SAN cells that control the heart rhythm. However, in tissue simulations, the mutations not only slowed down pacemaking, but also compromised AP conduction across the SAN-atrium, leading to a possible SAN exit block or sinus arrest, the major features of SSS. Simulated vagal nerve activity amplified the bradycardiac effects of the mutations. Two groups of SCN5A mutations showed subtle differences in impairing the ability of the SAN to drive the surrounding atrium, primarily attributable to their differential effects on atrial excitability and conduction safety. Experimental data with tetrodotoxin and carbachol confirmed the simulation outcomes.

Conclusions: Our study substantiates the causative link between SCN5A gene mutations and SSS and illustrates mechanisms by which the mutations impair the driving ability of the SAN. (Circ Res. 2010;107:126-137.)

Key Words: sick sinus syndrome ▪ SCN5A mutation ▪ ion channels ▪ computer modeling

Sick sinus syndrome (SSS) denotes a collection of cardiac arrhythmias associated with dysfunction of the sinoatrial node (SAN) that commonly lead to disorders in cardiac rhythm and conduction. Such arrhythmias include intermittent sinus bradycardia, sinus arrest, sinus pause, slow SAN-atrium conduction, sinus exit block or alternating bradycardia and atrial tachycardia. The symptoms of SSS vary, with patients presenting with syncope, presyncope, palpitations, or dizziness. Many SSS patients have to be fitted with an electronic pacemaker.

Mechanisms underlying the pathogenesis for sinus node dysfunction in SSS patients are unclear. SSS can occur in elderly and pediatric patients. It can also occur in healthy people without any evident structural heart disease but with genetic defects. Recent studies have identified several gene mutations in congenital SSS patients. Among them are the SCN5A gene mutations that alter the structure of the pore-forming \(\alpha\)-subunit of the cardiac Na\(^+\) channel. In congenital SSS families, Benson et al identified several SCN5A mutants that include threonine in place of isoleucine at position 220 (T220I), leucine in place of proline at position 1298 (P1298L), and in-frame deletion (delF1617) mutations. Functional analysis of the expression of the wild-type (WT) and mutant Na\(^+\) channels in cultured mammalian cell line (tsA201) revealed that these mutations resulted in reduced \(I_{\text{Na}}\) current density, together with impaired fast inactivation (ie, a shift in the voltage-dependent steady-state inactivation curve to a more hyperpolarizing potential) in comparison to the WT.
Smits et al identified another SCN5A mutation associated with SSS: replacement of glutamine by lysine at position 161 (E161K). Functional analysis of the E161K mutation also revealed reduced $I_{Na}$ density. However, in contrast to T220I, P1298L, and delF1617 mutations, the E161K mutation caused a shift in the steady-state activation curve to a more depolarized potential. Although all these SCN5A mutations share in common a reduction in positive shift of voltage-dependent activation may have similar consequences in terms of reducing the $I_{Na}$ “window” current. However, they may modulate differently cardiac excitability and, thus, differentially affect the ability of the SAN to pace and drive the surrounding atrial muscle.

The loss-of-function of $I_{Na}$ caused by SCN5A mutations is also associated with other cardiac diseases that include Brugada syndrome and progressive cardiac conduction system disease. However, the mechanistic link between loss-of-function of $I_{Na}$ arising from these mutations and impaired SAN function has not been elucidated. Primarily, whether and how these mutations compromise the ability of the SAN to drive the surrounding atrial muscle has not been investigated, as the existing experimental studies are limited to molecular and ionic levels.

Furthermore, the role of Na channels in cardiac pacemaking is still controversial, because the channels are expressed in peripheral SAN cells, but either completely absent or dramatically less-expressed in central SAN cells which mainly control the cardiac rhythm.

Computational models provide a powerful way to study the functional effects of reduced $I_{Na}$ in initiation and conduction of the pacemaking action potential (AP), an emergent dynamic behavior of coordinated ion channels and intercellular electric coupling. Smits et al have shown that the E161K mutation slows down the pacemaking rate in a peripheral SAN cell model, and AP conduction in a 1D atrial strand model. Both effects were enhanced by acetylcholine (ACh), the neurotransmitter released by the vagal nerves. However, the mechanisms by which the loss-of-function of $I_{Na}$ slows down the heart rate in the intact SAN-atrium (in which the heart rate is mainly controlled by central SAN cells with little or no $I_{Na}$ rather than peripheral SAN cells) and impairs AP conduction from the SAN to the atrial tissue (ie, the ability of the SAN to drive the surrounding atrium) have not been fully elucidated. It is also unclear whether or not the 2 distinctive mutation groups (G1 and G2) exhibit similarity or difference in their functional impacts.

The aim of this study was to address these issues.

**Methods**

The consequences of the impaired $I_{Na}$ channel function caused by several SCN5A mutations (T220I, P1298L, delF1617, and E161K) in generating sinus node dysfunction were investigated by using (1) previously developed electrophysiologically detailed mathematical models of the central and peripheral SAN cells; (2) two-dimensional anatomic models of the intact SAN-atrium tissue, incorporating accurate single-cell models of the SAN and the right atrium (RA); and histologically reconstructed tissue geometry; and (3) multielectrode extracellular potential recordings of the activation pattern from isolated intact rabbit SAN-atrium tissue to verify model predictions.

A full list of the model equations and parameters used for the central and peripheral SAN cells and RA cells under control conditions, SCN5A mutation conditions, and ACh conditions, as well as 2D tissue model of the intact SAN-atrium, is provided in the Online Data Supplement, available at http://circres.ahajournals.org (electronic copies of the respective codes are also available on request).

**Two-Dimensional Slice Model**

The 2D model of intact SAN-atrium tissue used in this study was based on histologically reconstructed geometry of a single slice of the rabbit RA, which was cut through the atrial muscle of the crista terminalis (CT) and the intercaval region with central and peripheral SAN areas (Figure 1A). The geometry presents a high spatial resolution (40 μm, which corresponds to 2 to 4 diameters of a cardiac myocyte) regular Cartesian grid of 210×45 nodes. For each node, a flag variable was used to identify whether it belongs to the SAN or RA cell type based on immunohistochemistry mapping data. The SAN was modeled by the Zhang et al equations for central and peripheral cells, and the RA was modeled by the Aslanidi et al equations for atrial cells, with each model producing different AP morphology (Figure 1A).

The model also incorporated an experimentally observed nonconductive region (“block zone”) next to the SAN toward the atrial septum, where cells have low excitability. The AP conduction in the 2D tissue model attributable to intercellular electric coupling via gap junctions was modeled through the diffusion coefficient, $D$. In the model, we considered the regional differences in the cellular electrophysiological properties and gap junctional coupling between the SAN center, periphery and atrial tissue as observed experimentally. A spatial gradient of $D$ (Figure 1F) was introduced as in our previous study. The gradient distributions of current densities from the central to the peripheral SAN were modeled by correlating them to cell membrane capacitance, $C_m$, which was assumed to be small in the center and large in the periphery. Spatial variations in all ion channel conductances were defined as a function of $C_m$ as found experimentally. To incorporate the effects of ACh into the cell models, (i) the ACh-activated K$^+$ current, $I_{K_{ACH}}$, was introduced, (ii) the L-type Ca$^{2+}$ current, $I_{L_{Ca,L}}$, was partially inhibited, and (iii) the activation curve of the hyperpolarization-activated current, $I_h$, was shifted toward more negative potentials. Full equations are presented in the Online Data Supplement.

The single-cell models for central and peripheral SAN cells and RA cells have been validated in our previous studies. The developed 2D anatomic model of the intact SAN-atrium was validated by its ability to reproduce the correct sequence of AP.

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initiation and conduction through the rabbit SAN-atrium, as observed experimentally.\textsuperscript{20,21} In the 2D model, the pacemaking AP was first initiated in the center of the SAN (Figure 1B and 1C), and it then propagated preferentially toward the CT; AP conduction toward the atrial septum was relatively slow. The space–time plot of the AP profiles recorded from cells along a line across the SAN-atrium (Figure 1D) showed continuous AP initiation and conduction. The computed activation time of the CT (1.5-mm distance from the center of the SAN) matched quantitatively to experimental data (Figure 1C), which validates the intercellular electric coupling used in the model. Simulation results with the 2D model were also validated against respective experimental data from isolated rabbit SAN-atrium (see below).

SCN5A Mutation Model

To model SCN5A mutations from G1 (T220I, P1298L, and delF1617), parameters of the fast Na\textsuperscript{+}/H\textsuperscript{+} current, \(I_{Na}\), were changed to reproduce experimental data (Figure 2). Note that prior experimental data\textsuperscript{2} were recorded from tsA201 cells transfected with a recombinant human Na\textsuperscript{+}/H\textsuperscript{+} channel cDNA (hH1), whereas our models are for the native current in rabbit SAN cells. Thus, in simulations, we shifted the steady-state inactivation curve by the same amount as observed experimentally (Figure 2C and 2D) and implemented the same percentage changes of the fast- and slow-inactivation time constants as observed experimentally (Figure 2A). We scaled the maximal \(I_{Na}\) channel conductance by a factor \(S_{CD}\) (see Online Table I) to reproduce the normalized current–voltage relationship (Figure 2E) and the reduction in \(I_{Na}\) as observed experimentally (Figure 2F). The heterozygous E161K mutation was modeled by the same method as used by Smits et al:\textsuperscript{6} \(I_{Na}\) was divided into 2 components: one for the WT \(I_{Na}\) and another for the E161K mutant \(I_{Na}'\). The WT component had a maximum conductance of 50\% of control, whereas the mutant component had a maximum conductance of 20\% of control. The steady-state activation curve of the mutant component was also shifted by 11.9 mV (Figure 2F). Full equations are presented in the Online Data Supplement.

Isolated Tissue Experiments

Five male adult rabbits (2 to 3 months old) were used for experimental study. The sinoatrial preparations were set up as described previously from animals killed by anesthetic overdose with venous injection of sodium phenobarbital (in accord with UK Home Office Legislation).\textsuperscript{22} After excision of the SAN and surrounding atrial muscle, the preparation was placed endocardial surface upwards in a tissue bath and superfused with modified Tyrode’s solution (in mmol/L: NaCl 120, NaHCO\textsubscript{3} 25.2, NaH\textsubscript{2}PO\textsubscript{4} 1.2, MgCl\textsubscript{2} 1.3, glucose 5, KCl 4.0, CaCl\textsubscript{2} 1.8, gassed with 95\% O\textsubscript{2}/5\% CO\textsubscript{2} at 37\°C and at a flow rate of \(\approx\)5 mL/min. Electric signals were obtained from the surface of this preparation by apposition of a custom-made extracellular multielectrode array that allowed electrograms to be monitored at multiple sites in the tissue as excitation passed under the array. The electrode array held 30 separate silver electrodes in a 5\%6 configuration (a detailed description of the electrodes is presented in the Online Data Supplement). The 30 recording electrodes were connected through shielded wires to a 32-channel amplifier (SCXI-1102C, National Instruments Corporation UK Ltd, Newbury, UK). The sampling frequency for each channel was set at 1 kHz. The signals were continuously sampled and stored on disk and displayed on screen using a custom-developed program, written in Labview 7.0 (National Instruments Corporation UK Ltd). Experiments were performed under the conditions of control, application of tetrodotoxin (TTX) (0.5 \(\mu\)mol/L; IC\textsubscript{50} \(\approx 0.1 \mu\)mol/L\textsuperscript{23} or a nondegrading ACh equivalent, carbachol (CCh) (200 mmol/L; IC\textsubscript{50} \(\approx 100 \mu\)mol/L\textsuperscript{24}), and a combination of TTX (0.5 \(\mu\)mol/L) and
CCh (200 nmol/L). Propagation maps were then derived during off-line analysis. The signals were displayed on screen in sets of 8 to 16 electrograms. The activation time was denoted as the point of maximal negative slope and marked with a cursor. After marking all significant waveforms in all leads, the activation times were then displayed in a grid representing the layout of the original recording array. All activation times, in milliseconds, were related to the timing of the first detected waveform.

Results

Mutation Effects on Single Cells

At the single-cell level, the central and peripheral SAN cell models were used to investigate the functional effects of the SCN5A mutations (G1: T220I, P1298L, and delF1617; G2: E161K) on AP generation under control and ACh conditions. Figure 3A through 3D shows effects of the P1298L mutation, and Figure 3E summarizes quantitative effects of all mutations. Figure 3A through 3D shows simulated AP with the WT and P1298L mutant channels, along with major underlying ionic currents in the central (left graphs) and peripheral (right graphs) cells. These simulations illustrate that the P1298L mutation did not affect the central cell (Figure 3A, i), but it dramatically slowed down pacemaking in the peripheral cell (Figure 3A, ii); the pacemaking cycle length (PCL) increased from 170 to 204 ms, an increase of 19.7%. This is similar to experimental observations by Honjo et al,12 who reported that blocking \( I_{Na} \) with TTX did not affect central cells, but slowed pacemaking of peripheral cells in the rabbit SAN. In the model, the resultant slowing of the pacemaking was primarily attributable to the mutation-induced decrease in \( I_{Na} \) (Figure 3B, ii), because other major underlying currents (such as \( I_{Ca,L} \) and \( I_{Kr} \)) were not greatly affected (Figure 3C [ii] and 3D [ii]). Functional effects of other mutations were similar: there was a slowing of pacemaking in peripheral cells and an increase of the PCL by 7.1%, 10.8%, 19.1%, and 7.1% for the T220I, delF1617, and E161K mutations, respectively (Figure 3E, ii).

ACh Effects on Single Cells

Application of ACh slowed down pacemaking by increasing the PCL in both central and peripheral SAN cells, with a larger effect on the central cells.16 Simulating effects of \( 5 \times 10^{-8} \) mol/L ACh resulted in the PCL increasing from 338 ms to 429 ms.
activated outward repolarizing current, diastolic depolarization period, which counterbalances the ACh-activated outward repolarizing current, leading to a greater slowing of the diastolic depolarization. Such behavior was also observed for other SCN5A mutations. With the same 5×10⁻⁸ mol/L ACh concentration, the PCL in the peripheral cell model increased by 26.0%, 50.4%, 45.3%, and 23.4% with the T220I, P1298L, delF1617, and E161K mutations respectively, which is markedly greater than with the WT channel (13.7%).

The dose-dependent effects of ACh on pacemaking APs with the WT and mutant channels were also simulated. Figure 5 presents results obtained from the peripheral cell model for different ACh concentrations with the WT (Figure 5A, 5C, and 5E) and the P1298L mutant (Figure 5B, 5D, and 5F) channels. It shows that increase in ACh concentration led to a greater increase in PCL, and the negative chronotropic effect was amplified by the P1298L mutation at all ACh concentrations. At an ACh concentration of 15×10⁻⁸ mol/L (Figure 5E), the cell with the WT channel still exhibited pacemaking (though with prolonged PCL), but the cell with the mutant channel became quiescent. Figure 5G summarizes the simulated dose-dependent effect of ACh on pacemaking APs with the WT and various mutant channels. It shows that all the mutations shifted the dose dependence of the PCL leftward, indicating a more suppressive effect of ACh on SAN cells incorporating ‘mutant’ $I_{Na}$. The dose-dependent effects of ACh on pacemaking at the 2D tissue level are illustrated in Figure 5H.

Two-Dimensional Tissue Effects

We investigated further the functional consequences of the mutations on AP conduction across the intact SAN-atrium under control and ACh conditions. Results are shown in Figure 6, which presents the spatial (running horizontally) and temporal (running vertically) profiles of APs recorded from representative cells across the SAN-atrium model in Figure 1A. Figure 6A presents the normal conduction in the normal tissue (with the WT channel and no ACh). Simulation of ACh addition (1.5×10⁻⁸ mol/L) slowed down the pacemaking rate (ie, led to an increase in the PCL) (Figure 6B). Figure 6C shows the AP profile with the P1298L mutation, a representative of the G1 group. Without ACh, the mutation slowed down the pacemaking rate, but did not impair the ability of the SAN to drive the atrium. However, with ACh the SAN pacemaker activity was abolished (Figure 6D), and, thus, the SAN lost the power to drive the atrium. Figure 6E and 6F show the effects of the G2 E161K mutation. Without ACh, the mutation slowed down pacemaking and also produced a conduction block in the direction toward the atrial septum; however, the AP conduction in the direction toward the CT was sustained. With an ACh concentration of 1.5×10⁻⁸ mol/L, the SAN was able to generate spontaneous activity but failed to drive the surrounding atrium; conduction exit block occurred in both directions.

Detailed simulations were also performed to investigate the effects of the mutations and ACh on the characteristics of AP initiation and conduction in the intact SAN-atrium tissue model. Figure 7A presents the measured activation timing sequence across the tissue with the WT and E161K mutant channels, with and without ACh. In the model, simulated application of ACh or the E161K mutation alone increased the time required for the AP to propagate from the center of the SAN to the RA, ie, the SAN conduction time (Figure 7A). The E161K mutation alone resulted in AP conduction failure in the direction toward the atrial septum. Furthermore, when ACh addition was simulated, there was a dramatic delay in AP initiation and also conduction block both toward the CT and the septum. However, the P1298L mutation markedly delayed AP initiation (Figure 7B), but did not increase the SAN conduction time; addition of ACh completely abolished the AP.
The measured AP conduction velocity in SAN-atrium tissue simulations also reflected the above observations. In the normal tissue with the WT channel, addition of ACh reduced the conduction velocity by \( \approx 12\% \) in the SAN (on average) and by \( \approx 7\% \) in the RA (Figure 7C). In tissue with the E161K mutant channel, the conduction velocity was decreased by \( \approx 46\% \) in the SAN (on average) and by \( \approx 45\% \) in the RA (Figure 7C). Subsequent addition of ACh to the tissue with the E161K mutant channel further reduced the AP conduction velocity in the SAN, and resulted in a complete conduction block in the atrium. In tissue with the P1298L mutant channel, the AP conduction velocity was decreased by \( \approx 8\% \) in the SAN (on average), and by \( \approx 8\% \) in the RA (Figure 7D); subsequent addition of ACh abolished pacemaking (Figure 7D).

Changes in the measured conduction velocity across the SAN-atrium were attributed to the changed maximum upstroke velocity \( (dV/dt_{\text{max}}) \), as shown in Figure 7E and 7F. In the normal tissue with the WT channel, simulated addition of ACh reduced \( dV/dt_{\text{max}} \) in both the RA and the SAN, with a greater effect on the RA. In tissue with the E161K mutant channel, there was a decrease in \( dV/dt_{\text{max}} \) throughout the tissue (up to \( \approx 27\% \)), not only in the RA and periphery of the SAN, but also in the center of the SAN; subsequent addition of ACh further reduced \( dV/dt_{\text{max}} \) in the SAN, with more dramatic effects in the SAN periphery, primarily at the border between the SAN and the atrium. This resulted in the SAN failing to drive the atrium, leading to SAN exit block. The P1298L mutation reduced \( dV/dt_{\text{max}} \) markedly in the SAN region, but only slightly in the RA region. Addition of ACh

![Figure 4. Effects of ACh on the SAN pacemaking rate. Simulated voltage and current recordings from central (i) and peripheral (ii) SAN cells. Effects of the SCN5A mutations and [ACh]=1.5×10^{-8} \text{ mol/L on APs (A), } I_{\text{K,ACh}} \text{ (B), } I_{\text{Ca,L}} \text{ (C), } I_{\text{f}} \text{ (D), } I_{\text{Na}} \text{ (E), and PCL (F) are shown.}](image-url)
to tissue with the P1298L mutant channel (Figure 7F) abolished pacemaking and \( \frac{dV}{dt} \) was zero throughout the SAN (data not shown).

**Effects on Excitability and Conduction Safety**

We have measured the excitation threshold, a reciprocal measure of cardiac excitability, for atrial cells in WT and mutant cases. Both the G1 and G2 mutations reduced atrial excitability, but to different extents. For the P1298L and E161K mutations, the measured excitation threshold increased from 1.7 to 2.1 and 2.8 nA, respectively, for a stimulus pulse duration of 1 ms. With simulated ACh addition, the respective changes were from 2.8 with the WT channel to 3.0 and 4.1 nA with the P1298L and E161K mutant channels. Thus, both mutations and ACh increased the excitation threshold for atrial cells. The larger increase of the excitation threshold associated with the G2 group (E161K) was correlated with higher vulnerability of the tissue to AP conduction block at the junction of the SAN-atrium (see Online Figure I).

Large differences in the magnitude of the threshold increase can be explained by the different nature of the mutations: although both the P1298L and E161K mutations result in about the same reductions in the current density of \( I_{\text{Na}} \) (Figure 2E and 2F), but the underlying kinetic changes are different. P1298L represents a family of mutations that cause hyperpolarizing shifts in the steady-state inactivation curve (Figure 2C), whereas E161K causes a depolarizing shift in the steady-state activation curve (Figure 2B). Both shifts decrease the window current, but only E161K substantially increased the excitation threshold, which is sensitive primarily to the activation kinetics of \( I_{\text{Na}} \). Results of a systematic
analysis of effects of activation and inactivation shifts on excitability are presented in the Online Data Supplement (see Online Figure I).

$\text{Na}^+$ channel defects that result in decreased tissue excitability have been associated with reduced AP conduction safety manifested as decreases in the safety factor (SF). Our simulations of SF changes (see the Online Data Supplement for details) attributable to effects of the SCN5A mutations and ACh suggested that the G2 (E161K) mutation results in a greater decrease of SF arising from greater decreased tissue excitability as compared to G1 mutations (Online Figure II).

Importantly, the AP conduction block attributable to the effects of the E161K mutation and high ACh concentration can be localized close to the exit site from the CT toward the RA (see Online Figure II), where low SF emerges from a combination of both electrophysiological (high excitation threshold, low $dV/dt_{\text{max}}$) and geometric (high source-to-load mismatch attributable to narrowing of the tissue) factors.

**Experimental Validation**

Multielectrode extracellular recordings of AP initiation and conduction pattern in isolated rabbit SAN-atrium tissue were performed experimentally to verify simulation results with the 2D tissue slice model (Figure 6). Because of lack of phenotypically accurate rabbit models, defective $I_{\text{Na}}$ due to the SCN5A variants was mimicked by reducing $I_{\text{Na}}$ through the application of TTX. Figure 8 shows the electric activation maps of the intact SAN-atrium tissue reconstructed from extracellular potentials recorded from the endocardial surface of the SAN-atrium preparation (see Methods). In control, the excitation was generated in the SAN center and propagated toward the RA with an average AP conduction velocity in the direction transverse to the CT of 0.12 m/s. In terms of the AP conduction velocity, application of 0.5 mol/L TTX alone slowed it down to 0.08 m/sec, whereas application of 200 nmol/L CCh alone did not have any substantial effect on the velocity. However, combined application of 0.5 mol/L TTX and 200 nmol/L CCh resulted in a much more substantial slowing of the AP conduction than TTX alone, down to 0.05 m/s, respectively. In terms of pacemaking rhythm, application of CCh or TTX alone resulted in an increase in the PCL from 340 to 380 ms, whereas combined application of TTX and CCh increased the PCL to 490 ms. The experimentally measured changes in both the AP conduction velocity and PCL are in good agreement with the simulation results (Figure 8), thereby supporting the predicted roles of SCN5A variants and the accentuating effects of ACh on impairing the cardiac pacemaker.

**Discussion**

In this study, we have developed a family of SAN cell models with WT and mutant SCN5A channels, and incorporated
them into an electrophysiologically and anatomically detailed 2D computer model of the rabbit SAN and surrounding atrium. Using the model, we investigated the functional effects of 2 groups of SCN5A mutations (G1 and G2) in impairing the ability of the SAN to pace and drive the surrounding atrial muscle. Functional differences between the 2 mutation groups were also studied. Our major findings are as follows: (1) At the intact SAN-atrium tissue level, $I_{Na}$ reduction attributable to the SCN5A mutations slowed down pacemaking and compromised the AP conduction across the SAN-atrium, leading to a possible sinus arrest or SAN exit block, which are major features of SSS. Our simulation results provide evidence substantiating the causative link between the identified gene mutations and familial SSS. (2) The functional impacts of reduced $I_{Na}$ at the isolated 

Figure 7. Effects of ACh and the SCN5A mutations on AP conduction parameters. Activation times with the E161K mutation and ACh (A) and the P1298L mutation (B) as compared to the WT channel; conduction velocities with the E161K mutation and ACh (C) and the P1298L mutation (D) as compared to the WT channel; $dv/dt_{max}$ with the E161K mutation and ACh (E) and the P1298L mutation (F) as compared to the WT channel. [ACh] = 1.5 x 10^{-6} mol/L.

Figure 8. Experimental validation of the SAN-atrium tissue model. Isolated SAN-atrium tissue with superimposed activation maps (top) show increase of the conduction time from the SAN into the RA caused by the effects of TTX, CCh, and their combination (see panel labels). AP conduction velocities and PCLs under all conditions considered match the respective values simulated with the 2D tissue model (bottom). IVC indicates inferior vena cava; SVC, superior vena cava. The leading pacemaker site of the SAN in control is shown with an asterisk.
SCN5A Mutations and Pacemaker Abnormalities

Our study has shown that, at the isolated single-cell level, all the considered SCN5A mutations slowed pacemaking of peripheral SAN cells, but not central SAN cells that normally initiate and control the heart rhythm. However, at the intact SAN-atrium tissue level, the SCN5A mutations slowed down the heart rate by slowing down pacemaking in the central cells, which have no \( I_{\text{Na}} \). This can be attributed to the electrotonic interaction between the SAN and the atrium. Because of a hyperpolarized resting potential (more negative than the maximal diastolic potential of the SAN) and lack of pacemaking activity, the atrium acts as an electric load suppressing the pacemaker activity of the SAN. Such a suppressive action can be mapped to the center of the SAN through cell-to-cell electric coupling, although its effects here are weaker than in the periphery. With the mutations, the suppressive action becomes greater as less \( I_{\text{Na}} \) is available to counterbalance the suppressive effect of adjacent atrial tissue.\(^{28}\)

Our simulations also showed that the mutations slowed AP conduction across the SAN-atrium. As shown by the activation time profiles (Figure 6), the AP conduction through the SAN is slow with all mutations, as often reported in SSS patients or patients with other cardiac conditions linked to mutations in the SCN5A gene.\(^{6,10}\) This is considered a sign of potential conduction failure risks, eg, sinus exit block.\(^1\) Conduction block was observed in our simulations with the E161K mutation (Figure 6E), in agreement with clinical observations.\(^6\) The block can be accounted for by a combination of decreased atrial excitability (Online Figure 1) and conduction safety (Online Figure II), which are most prominent with the E161K mutation.

ACh Effects

Addition of ACh slowed pacemaking in both the single-cell and 2D simulations, as did CCh in experiments; the negative chronotropic effect of ACh was greater with the SCN5A mutant channels than with the WT channel. The augmented bradycardiac effect of ACh can be attributed to the associated reduction of \( I_f \) (Figure 4). Without ACh, reduced \( I_{\text{Na}} \) leads to a more hyperpolarized membrane potential in SAN cells, which activates more \( I_f \). This increase in \( I_f \) counterbalances the reduced \( I_{\text{Na}} \) in the diastolic phase. However, as \( I_f \) is reduced by ACh, its counterbalancing effect on \( I_{\text{Na}} \) is also reduced, which results in the observed augmented negative chronotropic effect.

Note that cholinergic receptor stimulation by ACh also reduces \( I_{\text{Ca,L}} \), the effect ascribed to a protein kinase A–dependent reduction in L-type \( \text{Ca}^{2+} \) channel phosphorylation, which can occur either during \( \beta \)-adrenergoreceptor stimulation,\(^{29}\) or in its absence.\(^{30}\) However, it remains controversial whether ACh inhibitory effects on \( I_{\text{Ca,L}} \) do occur in the absence of concurrent \( \beta \)-adrenoreceptor stimulation. Recent findings\(^{24}\) have shown that in the absence of \( \beta \)-adrenergic stimulation CCh reduced the \( I_{\text{Ca,L}} \) amplitude by 20%, but only at a 10-fold higher [CCh] than that required to stop pacemaking; effects of lower [CCh], corresponding to physiologically feasible ACh levels, were much smaller. In our model, the description of the dose dependence of \( I_{\text{Ca,L}} \) on [ACh] has been based on similar data.\(^{16,30}\) Consequently, modulation of \( I_{\text{Ca,L}} \) by "physiological" [ACh] considered in our study is weak; removing this modulation from the model has not changed qualitative results of our simulations (quantitative changes to the pacemaking rate and AP conduction velocity were <10%).

Previous Modeling Studies

Note that our study of the conditions of compromised AP conduction in the SAN-atrium tissue and their mechanistic links with the SSS is related to, but distinct from, previous results by Kurata et al.\(^{31}\) That study focused on bifurcation structures of several single-cell models: (1) SAN cells under...
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used by Smits et al,6 with... Such mutations can be modeled by the same method as... been recorded from patients with heterozygous SCN5A mutations. Such mutations can be modeled by the same method as... modeling in situations where complete data from a single species... data are not unusual; this is a common practice in cardiac... phenotypically accurate rabbit models, in our in vitro experiments... feature of the present study is that, because of the lack of... occurring in interaction between the center and periphery of the SAN during pacemaking and following AP conduction from the SAN across the CT into the RA. These phenomena can be studied within a slice, where main 3D geometric features of the SAN-atrium tissue are projected onto a 2D plane transversal to the CT. That our approach using a 2D slice is reasonable is borne out by experimental data verifying our simulation results (Figure 8).

Mechanisms of SSS

Our simulations show that different mutations of the same gene (SCN5A) can lead to different modes of SAN dysfunction: abnormally slow pacemaking (with all mutations; enhanced by ACh), failure of the SAN to pacemake (G1: with T220I, P1298L, and delF1617 mutations at high ACh concentrations), or sinus exit block (G2; with the E161K mutation). All of these behaviors are typical of SSS.4,5 Although an exaggeration of the normal ageing process through factors such as degenerative fibrosis could also be an important cause, it is clear that ion channel mutations underlie the familial disease.3 In addition to SCN5A mutations, other ionic channel defects (such as mutations in the HCN4 gene encoding the hyperpolarization-activated cyclic nucleotide gated channel generating the pacemaking current, I\(_{\text{f}}\)) can also cause SSS. In cases where such mutations lead to abnormalities in AP rate and conduction, computational techniques similar to those used here may be used to provide mechanistic links between ion channel mutations and SSS.

Acknowledgment

We thank Prof. Wim Lammers at UAE University for developing extracellular mapping electrodes.

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This work was supported by project grant BBS/B/1678X from the Biotechnology and Biological Sciences Research Council (UK), a Doctoral Training Account from the Engineering and Physical Sciences Research Council (UK), and a Wellcome Trust UK grant (WT/081809/Z/06/Z).

Disclosures

None.

References


6. Smits JP, Koopmann TT, Wilders R, Veldkamp MW, Opsteltof H, Bhuiyan ZA, Mannens MM, Balser JR, Tan HL, Bezzina CR, Wilde AA. A mutation in the human cardiac sodium channel (E161K) contributes to the SAN (eg, exact locations of the sinus exit block sites, including the established block zone toward the atrial septum), which may depend on details of the tissue spatial structure, 3D heterogeneity and anisotropy. The dimensions of our tissue model have been reduced from full 3D to 2D, because we were primarily interested in interaction between the center and periphery of the SAN during pacemaking and following AP conduction from the SAN across the CT into the RA. These phenomena can be studied within a slice, where main 3D geometric features of the SAN-atrium tissue are projected onto a 2D plane transversal to the CT. That our approach using a 2D slice is reasonable is borne out by experimental data verifying our simulation results (Figure 8).

Mechanisms of SSS

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Disclosures

None.

References


Novelty and Significance

What Is Known?
• Sick sinus syndrome (SSS) is a collection of cardiac arrhythmias associated with dysfunction of the cardiac primary pacemaker: the sinoatrial node (SAN).
• Recent studies have identified several gene mutations in congenital SSS patients. Among them are mutations of the SCN5A cardiac Na+ channel.
• It is still unclear how SCN5A mutations compromise the ability of the SAN to pace and drive the surrounding atrial muscle in SSS patients.

What New Information Does This Article Contribute?
• At the single-cell level, SCN5A mutations slow down pacemaking rates in peripheral, but not in central, SAN cells that control the heart rhythm.
• In the SAN-atrial tissue, the mutations not only slow down pacemaking but also slow down the conduction across the SAN-atrium, leading to a possible sinus arrest or SAN exit block, the major features of SSS.
• Vagal nerve activity amplifies the bradycardic effects of the SCN5A mutations; it also compromises the ability of the SAN to pace and drive the atrium, leading to a higher probability of sinus arrest or SAN exit block than with the mutations alone.

The role of SCN5A gene mutations in compromising the ability of the SAN to drive the surrounding atrial muscle in SSS patients has hitherto been unclear. We sought to address this issue using cell and tissue mathematical models. Our major findings are as follows. (1) Reduction of Na+ channel current caused by the SCN5A mutations slows down both pacemaking and conduction across the SAN-atrium, leading to a possible sinus arrest or SAN exit block, the major features of SSS. (2) The functional impacts of reduced Na+ current at the isolated single-cell level differ from those seen at the intact SAN-atrium tissue level, at which electrotonic interactions occur between the SAN and atrium. (3) The bradycardic effects of the SCN5A mutations are amplified by vagal activity, increasing the probability of sinus arrest and SAN exit block. (4) Simulation data were supported by experimental data from isolated rabbit SAN-atrium tissue. For the first time, these findings illustrate mechanisms by which the SCN5A gene mutations impair the driving ability of the SAN. Our results are consistent with clinical observations and may provide new insights into the mechanisms underlying high risk of cardiac arrest in SSS patients at night, when vagal tone is greater.
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Timothy D. Butters, Oleg V. Aslanidi, Shin Inada, Mark R. Boyett, Jules C. Hancox, Ming Lei and Henggui Zhang

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Supplemental Material

Mechanistic links between Na\(^+\) channel (SCN5A) mutations and impaired cardiac pacemaking in sick sinus syndrome

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METHODS

2D slice model

The dynamics of initiation and conduction of electrical APs in the heart can be modelled using the standard Hodgkin-Huxley-type partial differential equation:\(^1,^2\)

\[
\frac{\partial V}{\partial t} = \nabla \cdot (D \nabla V) - \frac{I_{\text{tot}}}{C_m},
\]

(1)

where \(V\) is the membrane potential, \(t\) time, \(\nabla\) the spatial gradient operator, \(D\) the diffusion coefficient that characterises electrotonic spread of voltage via gap junctions, \(C_m\) the cell membrane capacitance and \(I_{\text{tot}}\) the total ionic current. Electrophysiologically-detailed models for \(I_{\text{tot}}\) – and hence, single cell APs – have been developed for rabbit SAN\(^1\) and RA\(^2\) cells.

The 2D model of intact SAN-atrium tissue was based on histologically reconstructed\(^3\) geometry of a single slice of the rabbit RA, which was cut through the atrial muscle of the crista terminalis and the intercaval region with central and peripheral SAN areas (Fig. 1A). The geometry presents a high spatial resolution (40 \(\mu m\), which corresponds to 2 to 4 diameters of a cardiac myocyte) regular Cartesian grid of 210 \(\times\) 45 nodes. For each node, a flag variable was used to identify whether it belongs to the SAN or RA cell type based on immunohistochemistry mapping data.\(^3\) The SAN was modelled by the Zhang et al. equations\(^1\) for central and peripheral cells, and the RA was modelled by the Aslanidi et al. equations\(^2\) for atrial cells, with each model producing different AP morphology (Fig. 1A).

The 2D model also incorporated an experimentally observed non-conductive region (“block zone”) next to the SAN towards the atrial septum, where cells have low excitability. In the block zone, \(I_{\text{tot}}\) in Equation 1 was described as:

\[
I_{\text{tot}} \equiv I_B = G_m(V - V_R).
\]

(2)

Here \(I_B\) is the passive membrane current, \(G_m\) is the membrane conductance, and \(V_R\) the resting membrane potential. Values for \(G_m\) and \(V_R\) were assumed to correspond to the membrane conductance and resting potential of the surrounding atrial tissue.

The AP conduction in the 2D tissue model due to intercellular electrical coupling via gap junctions was modelled through the diffusion coefficient, \(D\). In the model, we considered the regional differences in the electrophysiological properties and gap junctional coupling between the SAN centre, periphery and atrial tissue as observed experimentally.\(^3\) A spatial gradient of \(D\) (Fig. 1F) was introduced as in our previous study: \(^4\)
\[ D(x) = D_c + D_p \left( \frac{1.0}{1.0 + e^{-0.5(x-x_1)}} + \frac{1.0}{1.0 + e^{0.5(x-x_2)}} \right), \]  

where \( x \) is the horizontal coordinate through the 2D slice, \( x_1 \) and \( x_2 \) approximately correspond to the positions of the SAN boundaries within the tissue, and \( D_c \) and \( D_p \) are the diffusion coefficients of central and peripheral SAN cells, respectively.

The 2D model also considered the regional differences in the cellular electrical properties of SAN cells. The gradient distributions of current densities from the central to the peripheral SAN were modelled by correlating them to cell membrane capacitance (\( C_m \)), which was assumed to be small in the centre and large in the periphery.\(^{1,4}\) The spatial gradient distribution of \( C_m \) (Fig. 1E) was modelled as:

\[ C_m(x) = C_m^c + C_m^p \left( \frac{1.0}{1.0 + e^{-0.5(x-x_1)}} + \frac{1.0}{1.0 + e^{0.5(x-x_2)}} \right), \]  

where \( C_m^c \) and \( C_m^p \) are the capacitances of central and peripheral SAN cells, respectively.

Spatial variations in all ion channel conductances were defined as a function of \( C_m \) as found experimentally:\(^1\)

\[ g_Z(x) = \frac{C_m(x) - C_m^c}{C_m^p - C_m^c} g_Z^c + \frac{C_m^p - C_m(x)}{C_m^p - C_m^c} g_Z^p, \]  

where \( g_Z(x) \) is the conductance of one of the currents \( Z \in \{ \text{Na, Ca, K, \ldots} \} \), and \( g_Z^c \) and \( g_Z^p \) are the respective conductances for central and peripheral SAN cells.

**ACh effects**

ACh decreases the heart rate by slowing down the spontaneous diastolic depolarization rate of the SAN. In order to incorporate the effects of ACh into the cell models, an ACh-activated K\(^+\) current, \( I_{K,ACh} \), was introduced as part of \( I_{tot}.\(^5\)

\[ I_{K,ACh} = g_{K,ACh} \left( \frac{[K^+]_o}{10 + [K^+]_o} \right) \left( \frac{V - E_K}{1 + e^{(V-E_K-140)/2.5RT}} \right), \]  

where \( g_{K,ACh} \) is the maximum channel conductance, \( [K^+]_o \) is the extracellular K\(^+\) concentration, \( E_K \) is the equilibrium potential for K\(^+\) and \( F, R \) and \( T \) have their usual meanings. The conductance, \( g_{K,ACh} \), is ACh dose-dependent, as follows:
\[
g_{K,ACh} = g_{K,ACh,max} j^k \frac{[ACh]^{n_{K,ACh}}}{K_{0.5,K,ACh}^{n_{K,ACh}} + [ACh]^{n_{K,ACh}}} ,
\]

where \(g_{K,ACh,max}\) is the maximum value of \(g_{K,ACh}\), \(j\) and \(k\) are inactivation variables, \([ACh]\) is the ACh concentration, \(n_{K,ACh}\) is the Hill coefficient, and \(K_{0.5,K,ACh}\) is the ACh concentration that produces half-maximal activation of \(g_{K,ACh}\). \(I_{K,ACh}\) is included in both the SAN and atrial cell models, with \(g_{K,ACh,max}\) described by the same spatial gradient function as the other ion channel conductances, see Equation 5.

ACh also partially inhibits the L-type Ca\(^{2+}\) current, \(I_{Ca,L}\), and shifts the activation curve of the hyperpolarization-activated current, \(I_f\), towards more negative potentials. The fractional block, \(b\), for \(I_{Ca,L}\) is given by:

\[
b = b_{max} \frac{[ACh]}{K_{0.5,Ca} + [ACh]} ,
\]

where \(b_{max}\) is the maximum fraction of \(I_{Ca,L}\) block, and \(K_{0.5,Ca}\) is the ACh concentration that produces a half maximal block of \(I_{Ca,L}\). The shift in \(I_f\) activation curve is given by:

\[
s = s_{max} \frac{[ACh]^{nf}}{K_{0.5,f}^{nf} + [ACh]^{nf}} ,
\]

where \(s_{max}\) is the maximum shift of the \(I_f\) activation curve, \(n_f\) is the Hill coefficient, and \(K_{0.5,f}\) is the ACh concentration that produces a half maximal shift of the activation curve of \(I_f\).

**SCN5A mutation model**

In order to model SCN5A mutations from G1 (T220I, P1298L and delF1617), parameters of the fast Na\(^+\) current, \(I_{Na}\), were changed to reproduce experimental data (see in Fig. 2). Note that prior experimental data\(^6\) were recorded from tsA201 cells transfected with a recombinant human Na\(^+\) channel cDNA (hH1), whereas our models are for the native current in rabbit SAN cells. In simulations, we shifted the steady-state inactivation curve by the same amount as observed experimentally (Figs. 2C and 2D) and implemented the same percentage changes of the fast- and slow-inactivation time constants as observed experimentally (Fig. 2A). We scaled the maximal \(I_{Na}\) channel conductance by a factor \(S_{CD}\) to reproduce the normalised I-V relationship (Fig. 2E) and the reduction in \(I_{Na}\) as observed experimentally (Fig. 2F).

For a mutant model, the \(I_{Na}\) equation becomes:

\[
I_{Na} = (S_{CD} \times g_{Na}) m^3 h [Na^+]_o \frac{F^2}{RT} e^{(V - E_{Na})F/RT} \frac{1 - e^{-V/FRT}}{e^{V/FRT} - 1} ,
\]

where \(g_{Na}\) is the maximum conductance, \(m\) and \(h\) are inactivation variables, \([Na^+]_o\) is the intracellular Na\(^+\) concentration, \(E_{Na}\) is the Na\(^+\) equilibrium potential, \(F\) is the Faraday constant, \(R\) is the gas constant, and \(T\) is the temperature. The factor \(S_{CD}\) scales the maximal \(I_{Na}\) conductance to reproduce the normalised I-V relationship.
with inactivation time constants:

\[
\tau_{h1} = S \left( \frac{3.717 \times 10^{-6} e^{-0.2815(V+17.11)}}{1+3.732 \times 10^{-3} e^{-0.3426(V+37.76)}} + 5.977 \times 10^{-4} \right),
\]

(11)

\[
\tau_{h2} = S \left( \frac{3.186 \times 10^{-8} e^{-0.6219(V+18.8)}}{1+7.189 \times 10^{-5} e^{-0.6683(V+34.07)}} + 3.556 \times 10^{-3} \right),
\]

(12)

and voltage-dependent steady-state inactivation function, \( h_{I_{Na}} \):

\[
h_{I_{Na}} = \frac{1}{1+e^{(V-V^*_{N_{161K}} +66.1)/6.4}},
\]

(13)

where \( V^*_{N_{161K}} \) (mV) is a shift in the inactivation curve.

The heterozygous E161K mutation was modelled by the same method as used by Smits et al.\textsuperscript{7} \( I_{Na} \) was divided into two components – one for the WT \( I_{Na} \) and another for the E161K mutant \( I_{Na} \). The WT component had a maximum conductance of 50\% of control, whereas the mutant component had a maximum conductance of 20\% of control. The steady-state activation curve of the mutant component was also shifted by 11.9 mV (see in Fig. 2B):

\[
m^3_{I_{Na}} = \frac{1}{1+e^{(V-11.9)/5.46}}.
\]

(14)

A full list of the model equations and parameters used for the SAN central and peripheral cells and RA cells is presented below (Models I-III and Tables I-III).

**Numerical algorithms**

The 2D anatomical PDE model of the intact SAN-atrium was solved using the explicit Euler method with a 5-node approximation of the Laplacian operator. In numerical simulations, the time step was 0.005 ms and space step 0.04 mm, which gave accurate numerical solutions.

**Safety factor**

Safety factor (SF) has been introduced as a simple index of the robustness of conduction in cardiac tissues: it is defined as the ratio of charge generated to charge consumed by a cell during its excitation.\textsuperscript{8} SF > 1 indicates successful conduction, with the fraction of the value exceeding 1 indicative of the margin of safety. When SF is close to 1, conduction is critical, and when SF < 1, propagation fails. The equation used to compute SF is:
\[ SF = \frac{\int I_c \, dt + \int I_{\text{out}} \, dt}{\int I_{\text{in}} \, dt}; A \mid t \in [t_{1\%}, t_{\text{max}}] \] (15)

Here, \( I_c \) is the capacitive current of the cell, \( I_{\text{out}} \) is the intercellular current that flows from the cell to its downstream neighbour and \( I_{\text{in}} \) is the intercellular current that flows into the cell from its upstream neighbour. The domain of integration, \( A \), can be defined as the period of time from the moment when \( dV/dt \) at the AP wavefront reaches 1\% of its maximum to the moment when \( V \) reaches its maximum.\(^9\)

**Isolated tissue experiments**

5 male adult rabbits (2-3 months old) were used for experimental study. The sino-atrial preparations were set up as described previously from animals killed by anesthetic overdose with venous injection of sodium phenobarbital (in accord with UK Home Office Legislation).\(^10\) After excision of the SAN and surrounding atrial muscle, the preparation was placed endocardial surface upwards in a tissue bath and superfused with modified Tyrode's solution (in mmol/L: NaCl 120, NaHCO\(_3\) 25.2, NaH\(_2\)PO\(_4\) 1.2, MgCl\(_2\) 1.3, glucose 5, KCl 4.0, CaCl\(_2\) 1.8, gassed with 95\% O\(_2\)/5\% CO\(_2\)) at 37 °C and at a flow rate of ~5 ml/min. Electrical signals were obtained from the surface of this preparation by apposition of a custom-made extracellular multi-electrode array that allowed electrograms to be monitored at multiple sites in the tissue as excitation passed under the array. The electrode array held 30 separate silver electrodes in a 5×6 configuration. The inter-electrode distance was 0.55 mm; thus, the total array dimensions were approximately 3 mm length and 4 mm width. The electrodes were Teflon-coated silver wires with a coated outer diameter of 0.2 mm and an inner diameter of 0.125 mm (Science Products, Millville, NJ, USA). The spacing of the electrodes was obtained with 0.33 Teflon coated silver wires. The silver tips were chlorided. The 30 recording electrodes were connected through shielded wires to a 32-channel amplifier (SCXI-1102C, National Instruments Corporation UK Ltd, Newbury, UK). The sampling frequency for each channel was set at 1 kHz. The signals were continuously sampled and stored on disk and displayed on screen using a custom-developed program, written in Labview 7.0 (National Instruments Corporation UK Ltd, Newbury, Berks RG14 2PS, UK). Experiments were performed under the conditions of control, application of tetrodotoxin (TTX) (0.5 µmol/L; IC\(_{50}\) ~ 0.1 µmol/L) or a non-degrading ACh equivalent – carbachol (CCh) (200 nmol/L; IC\(_{50}\) ~ 100 nmol/L), and a combination of TTX (0.5 µmol/L) and CCh (200 nmol/L). Propagation
maps were then derived during off-line analysis. The signals were displayed on screen in sets of 8 to 16 electrograms. The activation time was denoted as the point of maximal negative slope and marked with a cursor. After marking all significant waveforms in all leads, the activation times were then displayed in a grid representing the layout of the original recording array. All activation times, in milliseconds, were related to the timing of the first detected waveform. Areas of isochronal activation in steps of 5 ms were plotted using the standard visualization package Paraview 6.0.

RESULTS

Effects on excitability
The G1 and G2 groups of SCN5A mutations modulate atrial excitability differently: one through shifting the steady-state inactivation curve to more negative potentials with the other shifting the steady-state activation curve to more positive potentials. Therefore, a systematic analysis of effects of activation and inactivation shifts on excitability and conduction was undertaken, the results of which are shown in Online Fig. I. Online Fig. IA, B shows plots of conduction velocity in a 1D strand of atrial cells against a range of inactivation shifts towards more negative potentials (Online Fig. IA), and a range of activation shifts towards more positive potentials (Online Fig. IB). In both cases, with the increase of the shift (0 corresponds to the WT channel), the measured conduction velocity monotonically decreased until conduction failed. This occurred at a \(-14\) mV shift in inactivation \(V_{0.5}\) and a \(7\) mV shift in activation \(V_{0.5}\). Such a difference in the ‘cut-off’ shift values for these two cases can be explained by differential change of the excitation threshold; this is shown in Online Fig. IC, D. These panels show plots of excitation threshold (measured as the minimal amplitude of an external current stimulus that provokes an atrial action potential) against a shift in steady-state inactivation (Online Fig. IC) or activation (Online Fig. ID). In both cases, a linear increase in the shift resulted in a monotonic increase in the measured excitation threshold. However, with the same value, the G2 shift always increased more the measured excitation threshold than the G1 shift (compare Online Figs. ID and IC). For the G1 case (Online Fig. IC), the excitation threshold increased from ~1.55 nA with the WT channel to ~1.65 nA when the inactivation curve was shifted by \(-14\) mV; for the G2 case (Online Fig. ID), the threshold increased from ~1.55 nA with the WT channel to ~2 nA when the activation curve was shifted by \(+7\) mV – for larger shift values the threshold kept increasing, and although APs could still be initiated.
in a single cell, their conduction in the tissue failed. Simulated addition of $1.5 \times 10^{-8} \text{ mol/L}$ ACh enhanced the effects described above, further decreasing the conduction velocities (Online Figs. IA and IB) and increasing the excitation thresholds (Online Figs. IC and ID) for both G1 and G2 mutations. This combination of effects can account for the higher susceptibility to conduction block in the 2D mutant tissues with ACh (see Fig. 6).

**Effects on conduction safety**

We further studied the effects of the SCN5A mutations and ACh on SF in the 2D model of SAN-atrium tissue. Online Fig. II illustrates SF distributions in the 2D tissue during AP conduction from the SAN into the RA. For the WT condition, SF was greater than 1 in all regions of the tissue, indicating safe conduction. Note that slightly smaller values of SF in the crista terminalis, as compared to the rest of the RA, were due to higher source-to-load mismatch within this thick bundle (large integral $I_m$ flowing from the SAN, relatively small integral $I_{out}$ flowing out to the RA). SCN5A Na$^+$ channel mutations for both the G1 (P1298L) and G2 (E161K) groups resulted in decreases throughout the tissue in the AP conduction velocity, $dV/dt_{\text{max}}$ (see in Fig. 7) and SF (Online Fig. II), all of which were decreased greater in the G2 (E161K) group than in the G1 (P1298L) group. Simulated addition of $1.5\times10^{-8} \text{ mol/L}$ ACh reduced SF in both WT and mutant cases. Again, reduction in SF was relatively greater in the G2 (E161K) mutation condition – in the region close to the exit-site from the crista terminalis into the RA, SF became less than 1 (Online Fig. II) leading to a conduction block in this direction (see in Fig. 6). Note that an apparent localised increase of SF within the crista terminalis in case of the E161K mutation was due to a large decrease of the AP conduction velocity (see in Fig. 7) – such a decrease allowed a time delay sufficient to balance out the source-to-load mismatch within this region. However, even though a source-to-load mismatch close to a narrow exit site from the crista terminalis towards the RA was similarly reduced, a substantial decrease of $dV/dt_{\text{max}}$ in this region due to the combination of the E161K mutation and ACh (Fig. 7) resulted in a large decrease of the capacitive current $I_c = C_m dV/dt$ – leading to a large decrease of SF and the resultant AP conduction block in this direction (see in Fig. 6 and Online Fig. II). The results of these simulations suggest that (i) the G2 (E161K) mutation results in the largest decrease of SF due to the largest decreases of $dV/dt_{\text{max}}$ and the AP conduction velocity as compared to the G1 SCN5A mutations (Figs. 7 and Online Fig. II); (ii) the AP conduction block due to the combination of the E161K mutation and high ACh concentration emerges from a decrease of SF localized close to the
exit-site from the crista terminalis towards the RA (Online Fig. II), where both the source-to-load mismatch due to narrowing of the RA tissue and the reduction of $dV/dt_{\text{max}}$ are substantial.

References
Online Table I. Values for mutation parameters.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>$S_{CD}$</th>
<th>$S_h$</th>
<th>$S_{\tau_1}$</th>
<th>$S_{\tau_2}$</th>
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<tr>
<td>T220I</td>
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<tr>
<td>P1298L</td>
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<tr>
<td>DelF1617</td>
<td>0.38</td>
<td>0.128</td>
<td>2.18</td>
<td>2.75</td>
</tr>
</tbody>
</table>

Model I. General single cell equations

$$\frac{dV}{dt} = -\frac{I_{ba}}{C_m}$$

Model I.1. Equilibrium potentials

$$E_{Na} = \frac{RT}{F} \ln \left[ \frac{[Na^+]_o}{[Na^+]_i} \right], \quad E_K = \frac{RT}{F} \ln \left[ \frac{[K^+]_o}{[K^+]_i} \right], \quad E_{Ca} = \frac{RT}{2F} \ln \left[ \frac{[Ca^{2+}]_o}{[Ca^{2+}]_i} \right], \quad E_{Cl} = \frac{RT}{F} \ln \left[ \frac{[Cl^-]_o}{[Ca^{2+}]_i} \right]$$

Model II. SAN cell model

$I_{tot} = I_{Na} + I_{Ca,L} + I_{Ca,T} + I_{to} + I_{sus} + I_{K,T} + I_{K,s} + I_f + I_{K,b} + I_{Na,b} + I_{Ca,b} + I_{NaCa} + I_{NaK} + I_{ACh}$

Model II.1. Fast Na$^+$ current

$$I_{Na} = g_{Na} m^3 h [Na^+]_o \frac{VF}{2} e^{\frac{(V-E_{Na}) F}{RT}} \frac{e^{\frac{VF}{RT}} - 1}{e^{\frac{VF}{RT}} - 1}$$

$$\frac{dm}{dt} = \frac{m_{\infty} - m}{\tau_m}$$

$$m_{\infty} = \left( \frac{1}{1 + e^{V/5.46}} \right)^{1/3}, \quad \tau_m = 1000 \left( \frac{0.6247 \times 10^{-2}}{0.832 e^{-0.335 (V + 56.7)} + 0.627 e^{0.082 (V + 65.01)}} + 4 \times 10^{-5} \right)$$

$$h = (1 - F_{Na}) h_1 + F_{Na} h_2, \quad F_{Na} = 9.52 \times 10^{-2} e^{-6.3 \times 10^{-7} (V + 34.4)} + 8.69 \times 10^{-2}$$

$$\frac{dh_1}{dt} = \frac{h_{1\infty} - h_1}{\tau_{h_1}}, \quad \frac{dh_2}{dt} = \frac{h_{2\infty} - h_2}{\tau_{h_2}}$$

$$h_{1\infty} = h_{2\infty} = \frac{1}{1 + e^{(V+66.1)/6.4}}$$

$$\tau_{h_1} = 1000 \left( \frac{3.717 \times 10^{-6} e^{-0.2815 (V + 17.11)}}{1 + 3.732 \times 10^{-3} e^{-0.342b (V + 37.76)}} + 5.977 \times 10^{-4} \right)$$

$$\tau_{h_2} = 1000 \left( \frac{3.186 \times 10^{-8} e^{-0.6219 (V + 18.8)}}{1 + 7.189 \times 10^{-5} e^{-0.6683 (V + 34.07)}} + 3.556 \times 10 \right)$$

Model II.2. L-type Ca$^{2+}$ current

$$I_{Ca,L} = g_{Ca,L} \left( d_L f_L + \frac{0.006}{1 + e^{-\frac{(V+14.1)/6.0}} (V - E_{Ca,L}) (1 - b)} \right)$$
\[
\frac{dd_L}{dt} = \frac{d_{L\infty} - d_L}{\tau_{d_L}},
\]
\[
d_{L\infty} = \frac{1.0}{1 + e^{-(V+35.0)/2.5}}, \quad \tau_{d_L} = \frac{1000.0}{\alpha_{d_L} + \beta_{d_L}},
\]
\[
\alpha_{d_L} = 14.19(V + 35.0) e^{-(V+14.1)/2.5} - 1, \quad \beta_{d_L} = 5.71(V - 5.0) e^{-(V-5.0)/2.5} - 1,
\]
\[
\frac{df_L}{dt} = \frac{f_{L\infty} - f_L}{\tau_{f_L}}.
\]
\[
f_{L\infty} = \frac{\alpha_{f_L}}{\alpha_{f_L} + \beta_{f_L}}, \quad \tau_{f_L} = \frac{1000.0}{\alpha_{f_L} + \beta_{f_L}},
\]
\[
\alpha_{f_L} = 3.12(V + 28.0) e^{-(V+28.0)/4.0} - 1, \quad \beta_{f_L} = 25.0 \left(1 + e^{-(V+28.0)/4.0}\right)^{4/3}.
\]
\[
b = b_{\max} \frac{[ACh]}{K_{0.5,ACh} + [ACh]}.
\]

**Model II.3. T-type Ca\(^{2+}\) current**

\[
I_{Ca, T} = \beta_g Ca, T \frac{d_T}{d (V - E_{Ca, T})},
\]
\[
\frac{dd_T}{dt} = \frac{d_{T\infty} - d_T}{\tau_{d_T}},
\]
\[
d_{T\infty} = \frac{1.0}{1 + e^{-(V+37.0)/6.8}}, \quad \tau_{d_T} = \frac{1000.0}{\alpha_{d_T} + \beta_{d_T}},
\]
\[
\alpha_{d_T} = 1068 e^{(V+26.3)/30.0}, \quad \beta_{d_T} = 1068 e^{-(V+26.3)/30.0},
\]
\[
\frac{df_T}{dt} = \frac{f_{T\infty} - f_T}{\tau_{f_T}}.
\]
\[
f_{T\infty} = \frac{1.0}{1 + e^{(V+71.7)/83.33}}, \quad \tau_{f_T} = \frac{1000.0}{\alpha_{f_T} + \beta_{f_T}},
\]
\[
\alpha_{f_T} = 15.3 e^{-(V+71.7)/83.33}, \quad \beta_{f_T} = 15 e^{(V+71.7)/15.38}.
\]

**Model II.4. Transient outward K\(^+\) current**

\[
I_{to} = g_{to} qr (V - E_K),
\]
\[
\frac{dr}{dt} = \frac{r_{\infty} - r}{\tau_r},
\]
\[
r_{\infty} = \frac{1.0}{1 + e^{-(V-10.93)/29.7}}, \quad \tau_r = 1000 \left(2.98 \times 10^{-3} + \frac{15.59 \times 10^{-3}}{1.037 e^{0.09 (V+30.61)} + 0.369 e^{0.12 (V+23.84)}}\right),
\]
\[
\frac{dg}{dt} = \frac{q_{\infty} - q}{\tau_{q_f}}.
\]
\[ q_{\infty} = \frac{1.0}{1 + e^{(V+59.37)/13.1}} \]
\[ \tau_q = 1000 \left( 10.1 \times 10^{-3} + \frac{65.17 \times 10^{-3}}{0.57 e^{-0.08 (V+498)} + 0.24 \times 10^{-4} e^{0.1(V+50.93)}} \right) \]

**Model II.5. Sustained outward current**

\[ I_{\text{sus}} = g_{\text{sus}} (V - E_{\text{sus}}) \]

**Model II.6. Fast delayed rectifier K\(^{+}\) current**

\[ I_{K,r} = g_{K,r} p_r (V - E_K) \]
\[ p_a = (1 - F_{K,r}) p_{a,f} + F_{K,r} p_{a,t} \]
\[ \frac{dp_{a,f}}{dt} = \frac{p_{a,in} - p_{a,f}}{\tau_{p_{a,f}}}, \quad \frac{dp_{a,t}}{dt} = \frac{p_{a,in} - p_{a,t}}{\tau_{p_{a,t}}} \]
\[ p_{a,in} = \frac{1.0}{1 + e^{-(V+14.2)/0.6}} \]
\[ \tau_{p_{a,f}} = 1000 \left( \frac{1.0}{37.2e^{(V-9)/15.9} + 0.96e^{-(V-9)/21.5}} \right), \quad \tau_{p_{a,t}} = 1000 \left( \frac{1.0}{4.2e^{(V-9)/15.9} + 0.15e^{-(V-9)/21.6}} \right) \]
\[ \frac{dp_i}{dt} = \frac{p_{i,in} - p_i}{\tau_{p_i}} \]
\[ p_{i,in} = \frac{1.0}{1 + e^{(V+18.6)/10.1}}, \quad \tau_{p_i} = 2.0 \]

**Model II.7. Slow delayed rectifier K\(^{+}\) current**

\[ I_{K,s} = g_{K,s} x_s^2 (V - E_K) \]
\[ \frac{dx_s}{dt} = \frac{x_{s,in} - x_s}{\tau_{x_s}} \]
\[ x_{s,in} = \frac{\alpha_{x_s}}{\alpha_{x_s} + \beta_{x_s}}, \quad \tau_{x_s} = \frac{1000.0}{\alpha_{x_s} + \beta_{x_s}} \]
\[ \alpha_{x_s} = \frac{14.0}{1 + e^{-(V-40)/9}}, \quad \beta_{x_s} = e^{-V/45} \]

**Model II.8. “Funny” current**

\[ I_f = I_{f,Na} + I_{f,K} \]
\[ I_{f,Na} = g_{f,Na} y(V - E_{Na}) \]
\[ I_{f,K} = g_{f,K} y(V - E_K) \]
\[ \frac{dy}{dt} = \frac{y_{in} - y}{\tau_y} \]
\[ y_{in} = \frac{\alpha_y}{\alpha_y + \beta_y}, \quad \tau_y = \frac{1000.0}{\alpha_y + \beta_y} \]
\[ \alpha_y = e^{-(V+78.91)/26.2}, \quad \beta_y = e^{(V+75.13)/21.25} \]
\[
s = s_{\text{max}} \frac{[\text{ACh}]^f}{K_{n,5.5}^f + [\text{ACh}]^f}
\]

**Model II.9. Na\(^+\)-Ca\(^{2+}\) exchanger current**

\[
I_{\text{NaCa}} = k_{\text{NaCa}} \frac{[\text{Na}^+]_i^n[\text{Ca}^{2+}]_o^n e^\gamma \gamma_{\text{NaCa},VF}/RT - [\text{Na}^+]_o^n[\text{Ca}^{2+}]_i^n e^{(\gamma \gamma_{\text{NaCa},-1})VF}/RT}{1 + d_{\text{NaCa}} ([\text{Na}^+]_i^n[\text{Ca}^{2+}]_o^n + [\text{Na}^+]_o^n[\text{Ca}^{2+}]_i^n)}
\]

**Model II.10. Na\(^+\)-K\(^+\) pump current**

\[
I_{\text{NaK}} = I_{\text{NaK}} \left( \frac{[\text{Na}^+]_i}{K_{m,\text{Na}} + [\text{Na}^+]_i} \right)^3 \left( \frac{[\text{K}^+]_o}{K_{m,k} + [\text{K}^+]_o} \right)^2 \left( 1.5 + e^{-(V+60)/40} \right)
\]

**Model II.11. Background currents**

\[
I_{\text{Na.b}} = g_{\text{Na.b}}(V - E_{\text{Na}}), \quad I_{\text{Ca.b}} = g_{\text{Ca.b}}(V - E_{\text{Ca}}), \quad I_{\text{K.b}} = g_{\text{K.b}}(V - E_{\text{K}})
\]

**Model II.12. Acetylcholine activated K\(^+\) current**

\[
I_{\text{K,ACH}} = g_{\text{K,ACH}} \left( \frac{[\text{K}]}{10 + [\text{K}]} \right) \left( 1 + e^{(V-E_{\text{K}}-140)/2.5RT} \right)
\]

\[
g_{\text{K,ACH}} = \frac{g_{\text{K,ACH}} jk}{K_{n,5.5,K,ACH}^f + [\text{ACh}]^f}
\]

\[
dj = j - j
\]

\[
dr = \frac{j}{\tau_j}
\]

\[
j = \frac{a_j}{a_j + \beta_j}, \quad \tau_j = \frac{1000.0}{a_j + \beta_j}
\]

\[
a_j = 73.1, \quad \beta_j = 120 \frac{1}{1 + e^{-(V+50)/15}}
\]

\[
dk = k - k
\]

\[
dr = \frac{k}{\tau_k}
\]

\[
k = \frac{a_k}{a_k + \beta_k}, \quad \tau_k = \frac{1000.0}{a_k + \beta_k}
\]

\[
a_k = 3.7, \quad \beta_k = 5.82 \frac{1}{1 + e^{-(V+50)/15}}
\]

**Online Table II. Model parameter values**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Center</th>
<th>Periphery</th>
</tr>
</thead>
<tbody>
<tr>
<td>(B_{\text{max}})</td>
<td>0.56</td>
<td>0.56</td>
</tr>
<tr>
<td>(C_m)</td>
<td>20 pF</td>
<td>65 pF</td>
</tr>
<tr>
<td>(D_{\text{NaCa}})</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>(E_{\text{Ca,l}})</td>
<td>46.4 mV</td>
<td>46.4 mV</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value</td>
<td>Value</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>$E_{Ca,T}$</td>
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<td>45 mV</td>
</tr>
<tr>
<td>$E_{sus}$</td>
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<td>-</td>
</tr>
<tr>
<td>$G_{Na}$</td>
<td>0 µS/pF</td>
<td>1.85 x 10^{-8} µS/pF</td>
</tr>
<tr>
<td>$G_{Ca,L}$</td>
<td>2.9 x 10^{-4} µS/pF</td>
<td>1.0 x 10^{-3} µS/pF</td>
</tr>
<tr>
<td>$G_{Ca,T}$</td>
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<td>2.14 x 10^{-4} µS/pF</td>
</tr>
<tr>
<td>$G_{io}$</td>
<td>2.5 x 10^{-4} µS/pF</td>
<td>5.6 x 10^{-4} µS/pF</td>
</tr>
<tr>
<td>$G_{sus}$</td>
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<td>1.8 x 10^{-4} µS/pF</td>
</tr>
<tr>
<td>$G_{K,ACH}$</td>
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<td>1.218 x 10^{-9} µS/pF</td>
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<tr>
<td>$G_{K,r}$</td>
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<tr>
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<tr>
<td>$G_{K1}$</td>
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<td>-</td>
</tr>
<tr>
<td>$G_{i,Na}$</td>
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<td>1.05 x 10^{-4} µS/pF</td>
</tr>
<tr>
<td>$G_{i,K}$</td>
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</tr>
<tr>
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<tr>
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<tr>
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<td>1.3 x 10^{-6} µS/pF</td>
</tr>
<tr>
<td>$G_{NaCa}$</td>
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<td>1.36 x 10^{-7} µS/pF</td>
</tr>
<tr>
<td>$[Na^+]_{o}$</td>
<td>140 mM</td>
<td>140 mM</td>
</tr>
<tr>
<td>$[Na^+]_{i}$</td>
<td>8 mM</td>
<td>8 mM</td>
</tr>
<tr>
<td>$[Ca^{2+}]_{o}$</td>
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<td>2 mM</td>
</tr>
<tr>
<td>$[Ca^{2+}]_{i}$</td>
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<td>0.0001 mM</td>
</tr>
<tr>
<td>$[K^+]_{o}$</td>
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<td>5.4 mM</td>
</tr>
<tr>
<td>$[K^+]_{i}$</td>
<td>140 mM</td>
<td>140 mM</td>
</tr>
<tr>
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<td>0.621</td>
</tr>
<tr>
<td>$K_{m,Na}$</td>
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<td>5.64</td>
</tr>
<tr>
<td>$K_{0.5, Ca}$</td>
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<td>0.09 µM</td>
</tr>
<tr>
<td>$K_{0.5,f}$</td>
<td>1.26 x 10^{-2} µM</td>
<td>1.26 x 10^{-2} µM</td>
</tr>
<tr>
<td>$K_{0.5,K, ACH}$</td>
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<td>0.28 µM</td>
</tr>
<tr>
<td>$N_{K,ACh}$</td>
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<td>1.5</td>
</tr>
<tr>
<td>$N_{f}$</td>
<td>0.69</td>
<td>0.69</td>
</tr>
<tr>
<td>$S_{max}$</td>
<td>-7.2 mV</td>
<td>-7.2 mV</td>
</tr>
<tr>
<td>$\Gamma_{NaCa}$</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Model III. Atrial cell model

\[ I_{\text{tot}} = I_{\text{Na}} + I_{\text{Ca,L}} + I_{\text{Ca,T}} + I_{\text{so}} + I_{\text{sus}} + I_{\text{K,e}} + I_{\text{K,s}} + I_{\text{K1}} + I_{\text{NaCa}} + I_{\text{NaK}} + I_{\text{Na,b}} + I_{\text{Ca,b}} + I_{\text{Ca,p}} \]

Model III.1. Fast Na\(^+\) current

\[ I_{\text{Na}} = g_{\text{Na}} m^3 h [\text{Na}^+]_o \frac{VF^2}{RT} \left( \frac{e^{(V-E_{\text{Na}})/RT} - 1}{e^{VF/RT} - 1} \right) \]

\[ \frac{dm}{dt} = \frac{m_{\infty} - m}{\tau_m} \]

\[ m_{\infty} = \frac{a_m}{\alpha_m + \beta_m}, \quad \tau_m = \frac{1000.0}{\alpha_m + \beta_m} \]

\[ a_m = \frac{460.0(V + 44.4)}{1 - e^{-(V + 44.4)/12.673}}, \quad \beta_m = 18400 \cdot 0.0 e^{-(V + 44.4)/12.673} \]

\[ h = 0.635 h_1 + 0.365 h_2 \]

\[ \frac{dh_1}{dt} = \frac{h_{1\infty} - h_1}{\tau_{h_1}} \quad \frac{dh_2}{dt} = \frac{h_{2\infty} - h_2}{\tau_{h_2}} \]

\[ h_{1\infty} = h_{2\infty} = \frac{a_h}{a_h + \beta_h} \]

\[ a_h = 44.9 e^{-(V + 66.9)/5.57}, \quad \beta_h = \frac{1491.0}{1 + 323.3 e^{-(V + 94.6)/12.9}} \]

\[ \tau_{h_1} = 1000 \left( \frac{0.03}{1 + e^{(V + 40.0)/6.0}} + 0.00015 \right) \]

\[ \tau_{h_2} = 1000 \left( \frac{0.12}{1 + e^{(V + 55.0)/2.0}} + 0.00045 \right) \]

Model III.2. L-type Ca\(^{2+}\) current

\[ I_{\text{Ca,L}} = g_{\text{Ca,L}} \left( \frac{d_L f_L + 1.0}{1 + e^{-(V - 23)/12.0}} \right) (V - E_{\text{Ca,L}}) \]

\[ \frac{dd_L}{dt} = \frac{d_{L\infty} - d_L}{\tau_{d_L}} \]

\[ d_{L\infty} = \frac{1.0}{1 + e^{-(V + 10.95)/6.6}}, \quad \tau_{d_L} = \frac{1000.0}{\alpha_{d_L} + \beta_{d_L}} \]

\[ a_{d_L} = \frac{16.72(V + 45.0)}{1 - e^{-(V + 45.0)/2.5}} + \frac{50.0(V + 10)}{1 - e^{-(V + 10)/4.808}} \]

\[ \beta_{d_L} = \frac{4.48(V + 5.0)}{e^{(V + 5.0)/2.5} - 1} \]

\[ \frac{df_L}{dt} = \frac{f_{L\infty} - f_L}{\tau_{f_L}} \]

\[ f_{L\infty} = \frac{a_{f_L}}{a_{f_L} + \beta_{f_L}}, \quad \tau_{f_L} = \frac{1000.0}{a_{f_L} + \beta_{f_L}} \]

\[ a_{f_L} = \frac{8.49(V + 18.0)}{e^{(V + 18.0)/4.0} - 1}, \quad \beta_{f_L} = \frac{67.922}{1 + e^{-(V + 18.0)/4.0}} \]

Model III.3. T-type Ca\(^{2+}\) current

\[ I_{\text{Ca,T}} = g_{\text{Ca,T}} d_f f_T (V - E_{\text{Ca,T}}) \]
\[
\frac{dd_T}{dt} = \frac{d_{T\infty} - d_T}{\tau_{dT}}, \\
\frac{df_T}{dt} = \frac{f_{T\infty} - f_T}{\tau_{fT}}, \\
\frac{df_{T\infty}}{dt} = \frac{\alpha_{fT}}{\alpha_{fT} + \beta_{fT}}, \\
\tau_{fT} = \frac{1000.0}{\alpha_{fT} + \beta_{fT}}, \\
\alpha_{fT} = 9.637e^{-(V+75.0)\beta \alpha}, \\
\beta_{fT} = 9.637e^{(V+75.0)/15.38}
\]

**Model III.4. Transient outward \(K^+\) current**

\[
I_{to} = g_{to} r \left(0.59s_1^3 + 0.41s_2^3\right) \left(0.6s_3^6 + 0.4\right)(V - E_K)
\]

\[
\frac{dr}{dt} = \frac{r_{infty} - r}{\tau_r}, \\
r_{infty} = \frac{1.0}{1 + e^{-(V+15.0)/5.633}}, \\
\tau_r = 1000 \left(\frac{1.0}{\alpha_r + \beta_r} + 0.0004\right), \\
\alpha_r = 386.6e^{V/12}, \\
\beta_r = 8.011e^{-V/7.2}
\]

\[
\frac{ds_1}{dt} = \frac{s_{1\infty} - s_1}{\tau_{s1}}, \\
\frac{ds_2}{dt} = \frac{s_{2\infty} - s_2}{\tau_{s2}}, \\
s_{1\infty} = s_{2\infty} = \frac{1.0}{1 + e^{(V+28.29)/7.06}}
\]

\[
\tau_{s1} = 1000 \left(\frac{0.189}{1 + e^{(V+32.8)/0.1}} + 0.0204\right), \\
\tau_{s2} = 1000 \left(\frac{0.189}{1 + e^{(V+32.8)/0.1}} + 0.45e^{-(V+13.54)/13.97}\right)
\]

\[
\frac{ds_3}{dt} = \frac{s_{3\infty} - s_3}{\tau_{s3}}, \\
s_{3\infty} = \frac{1.0}{1 + e^{(V+50.67)/27.38}} + 0.666, \\
\tau_{s3} = 1000 \left(\frac{7.5}{1 + e^{(V+23.0)/0.5}} + 0.5\right)
\]

**Model III.5. Sustained outward current**

\[
I_{sus} = g_{sus} (V - E_{sus})
\]

**Model III.6. Fast delayed rectifier \(K^+\) current**

\[
I_{Kr} = g_{Kr} p_a p_s (V - E_K)
\]

\[
\frac{dp_a}{dt} = \frac{p_{a\infty} - p_a}{\tau_{p_a}}
\]
\[ p_{an} = \frac{1.0}{1 + e^{-\left(V + 5.1\right)/13.8}}, \quad \tau_{\alpha_n} = \frac{1000.0}{\alpha_{p_n} + \beta_{p_n}} \]
\[ \alpha_{p_n} = 9.0 e^{-V/25.371}, \quad \beta_{p_n} = 1.3 e^{-V/13.026} \]
\[ \frac{dp_i}{dt} = \frac{p_{io} - p_i}{\tau_{p_i}} \]
\[ p_{io} = \frac{\alpha_{p_i}}{\alpha_{p_i} + \beta_{p_i}}, \quad \tau_{p_i} = \frac{1000.0}{\alpha_{p_i} + \beta_{p_i}} \]
\[ \alpha_{p_i} = 100.0 e^{-V/54.645}, \quad \beta_{p_i} = 656.0 e^{V/106.157} \]

**Model III.7. Slow delayed rectifier K\(^+\) current**

\[ I_{K,s} = g_{K,s} n(V - E_K) \]
\[ \frac{dn}{dt} = \frac{n_m - n}{\tau_n} \]
\[ n_m = \frac{1.0}{1 + e^{-\left(V + 0.9\right)/13.8}}, \quad \tau_n = 1000 \left( \frac{1.0}{\alpha_n + \beta_n} + 0.060 \right) \]
\[ \alpha_n = 1.66 e^{V/69.452}, \quad \beta_n = 0.3 e^{-V/21.826} \]

**Model III.8. Inward rectifier K\(^+\) current**

\[ I_{K1} = g_{K1} \left( \frac{[K^+]_0}{K_{m,K1} + [K^+]_0} \right)^3 \frac{V - E_K}{1 + e^{-1.593(V - E_K + 3.6)F/RT}} \]

**Model III.9. Na\(^+\)-Ca\(^{2+}\) exchanger current**

\[ I_{NaCa} = k_{NaCa} \left( \frac{[Na^+]_0^3 [Ca^{2+}]_0 e^{\gamma_{NaCa} V/RT} - [Na^+]_0^3 [Ca^{2+}]_0 e^{(\gamma_{NaCa} - 1) V/RT}}{1 + d_{NaCa} ([Na^+]_0^3 [Ca^{2+}]_0 + [Na^+]_0^3 [Ca^{2+}]_0)} \right) \]

**Model III.10. Na\(^+\)-K\(^+\) pump current**

\[ I_{NaK} = I_{NaK} \left( \frac{[Na^+]_0^{1.5}}{K_{m,Na}^{1.5} + [Na^+]_0^{1.5}} \right) \left( \frac{[K^+]_0}{K_{m,K} + [K^+]_0} \right) \frac{1.6}{1.5 + e^{-(V+660)/40}} \]

**Model III.11. Ca\(^{2+}\) pump current**

\[ I_{Ca,p} = \hat{I}_{Ca,p} \left( \frac{[Ca^{2+}]_0}{[Ca^{2+}]_0 + 0.0002} \right) \]

**Model III.12. Background currents**

\[ I_{Na,b} = g_{Na,b} (V - E_{Na}), \quad I_{Ca,b} = g_{Ca,b} (V - E_{Ca}) \]
### Online Table III. Model parameter values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{Na}$</td>
<td>$0.028 \times 10^{-6}$ µS/pF</td>
</tr>
<tr>
<td>$g_{Ca,L}$</td>
<td>$144.0 \times 10^{-6}$ µS/pF</td>
</tr>
<tr>
<td>$g_{Ca,T}$</td>
<td>$120.0 \times 10^{-6}$ µS/pF</td>
</tr>
<tr>
<td>$g_{lo}$</td>
<td>$200.0 \times 10^{-6}$ µS/pF</td>
</tr>
<tr>
<td>$g_{sus}$</td>
<td>$26.00 \times 10^{-6}$ µS/pF</td>
</tr>
<tr>
<td>$g_{K,r}$</td>
<td>$70.00 \times 10^{-6}$ µS/pF</td>
</tr>
<tr>
<td>$g_{K,s}$</td>
<td>$50.00 \times 10^{-6}$ µS/pF</td>
</tr>
<tr>
<td>$g_{K1}$</td>
<td>$203.2 \times 10^{-6}$ µS/pF</td>
</tr>
<tr>
<td>$g_{Na,b}$</td>
<td>$0.400 \times 10^{-6}$ µS/pF</td>
</tr>
<tr>
<td>$g_{Ca,b}$</td>
<td>$0.400 \times 10^{-6}$ µS/pF</td>
</tr>
<tr>
<td>$k_{NaCa}$</td>
<td>$0.400 \times 10^{-6}$ µS/pF</td>
</tr>
<tr>
<td>$I_{NaK}$</td>
<td>$1.288 \times 10^{3}$ nA/pF</td>
</tr>
<tr>
<td>$I_{Ca,p}$</td>
<td>$190.0 \times 10^{-6}$ µS/pF</td>
</tr>
<tr>
<td>$d_{NaCa}$</td>
<td>0.0003</td>
</tr>
<tr>
<td>$\gamma_{NaCa}$</td>
<td>0.4500</td>
</tr>
<tr>
<td>$K_{m,Na}$</td>
<td>11.00 mM</td>
</tr>
<tr>
<td>$K_{m,K}$</td>
<td>1.000 mM</td>
</tr>
<tr>
<td>$K_{m,K1}$</td>
<td>0.590 mM</td>
</tr>
<tr>
<td>$[Na^+]_o$</td>
<td>140.0 mM</td>
</tr>
<tr>
<td>$[Ca^{2+}]_o$</td>
<td>2.500 mM</td>
</tr>
<tr>
<td>$[K^+]_o$</td>
<td>5.000 mM</td>
</tr>
<tr>
<td>$E_{sus}$</td>
<td>-70 mV</td>
</tr>
<tr>
<td>$E_{Ca,L}$</td>
<td>50 mV</td>
</tr>
<tr>
<td>$E_{Ca,T}$</td>
<td>38 mV</td>
</tr>
<tr>
<td>$R$</td>
<td>8314 mJ/mol °C</td>
</tr>
<tr>
<td>$F$</td>
<td>96487 C/mol</td>
</tr>
<tr>
<td>$T$</td>
<td>35°C</td>
</tr>
</tbody>
</table>
Online Figure I. Effects of shifts in steady-state voltage-dependent inactivation/activation associated with G1/G2 mutations on atrial excitability. A, B: Conduction velocity in one-dimensional atrial tissue against a shift of the steady-state inactivation (A) or activation (B) curve. C, D: Excitation threshold of a single atrial cell against a shift of the steady-state inactivation (C) or activation (D) curve. [ACh] = 0 (closed circles) or [ACh] = 1.5 \times 10^{-8} mol/L (open circles).
Online Figure II. Effects of the SCN5A mutations and ACh on the AP conduction safety. Spatial distributions of the SF during AP conduction from the SAN into the RA in the 2D tissue slice model are colour-coded according to palette on the right; mutations (P1298L and E161K) and ACh concentrations are indicated in the respective labels.