Mechanism of Sinus Node Dysfunction in Carriers of the E161K Mutation in the *SCN5A* Gene

Ronald Wilders

Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Abstract

Heterozygous carriers of the E161K mutation in the SCN5A gene, which encodes the $Na_V1.5$ pore-forming α-subunit of the ion channel carrying the cardiac fast sodium current (I_{Na}), show sinus node dysfunction.

We assessed the mechanism by which the E161K mutation causes sinus bradycardia and reduces atrial excitability, as well as the potential role of the common H558R polymorphism. To this end, we incorporated reported mutation-induced changes in I_{Na} into the recently developed Fabbri-Severi model of a single human sinoatrial node (SAN) cell. The threshold current of the Courtemanche-Ramirez-Nattel human right atrial cell model was used as a measure of atrial excitability.

The E161K/H558 mutation significantly increased the cycle length of the SAN cell, in particular under vagal tone. The mutant component of I_{Na} was effectively zero, thus slowing diastolic depolarization. Highly similar results were obtained with the E161K/R558 mutation. The E161K mutation increased the threshold stimulus current of the atrial cell by a factor of ≈2.2, virtually independent of the H558 or R558 background.

We conclude that the E161K mutation underlies the clinically observed sinus node dysfunction. Furthermore, we conclude that the common H558R polymorphism does not significantly alter the effects of the E161K mutation.

1. Introduction

1.1. Aim of the study

The 'fast sodium current' (I_{Na}), which flows through $Na_V1.5$ sodium channels, is a key player in the electrical activity of the human heart [1]. The cardiac-specific $Na_V1.5$ protein, encoded by the *SCN5A* gene, is the pore-forming α-subunit of the channel. Of note, a functional I_{Na} channel is built with a single $Na_V1.5$ protein, in contrast with, for example, a functional 'pacemaker current' or 'funny current' (I_f) channel, which is a tetramer of four proteins from the HCN family, in particular HCN4 [2,3].

The large and fast influx of sodium ions through the $Na_V1.5$ channels is not only responsible for the fast upstroke of individual atrial and ventricular cardiomyocytes, but also for the fast impulse propagation in the atrial and ventricular tissue. Thus, I_{Na} is an important determinant of the PQ interval, or PR interval, and the QRS duration on the body surface ECG. I_{Na} has also been observed in human sinoatrial node cells [4]. Accordingly, it was included in the comprehensive computational model of a single human sinoatrial node (SAN) cell that was recently developed by Fabbri et al. [5].

Mutations in genes encoding ion channel-related proteins may result in inherited arrhythmia disorders, in particular the long QT syndrome (LQTS) and the Brugada syndrome (BrS) [6–8]. LQTS shows an estimated prevalence of 1:2,000 [9] and is the most commonly encountered inherited arrhythmia disorder in clinical practice (≈35% [10]). Estimates of the prevalence of BrS range from 1:5,000 to 1:2,000 [8].

LQTS type 3 (LQT3) is caused by gain-of-function mutations in the *SCN5A* gene. Slowed or incomplete inactivation of the $Na_V1.5$ channel results in an additional inward current, known as late or persistent I_{Na}, during the course of the ventricular action potential and thereby in an increase in action potential duration and prolongation of the QT interval on the ECG. The estimated prevalence of LQT3 among LQTS patients is ≈10% [11,12].

As far as a genetic basis for BrS is found, mutations in the *SCN5A* gene (BrS type 1, BrS1) are by far the most common, with a prevalence of up to 30% of all cases in which a genetic variant is implicated [8]. Mutations in *SCN5A* associated with BrS are all loss-of-function mutations, either caused by decreased expression of $Na_V1.5$ in the sarcolemma, expression of non-functional channels, or altered gating properties leading to a decreased I_{Na} (e.g., delayed activation or earlier or faster inactivation) [7]. The mechanistic link between the BrS1 channelopathies and the associated changes in the ECG pattern are still a matter of debate [7,8].

Mutations in *SCN5A* may also lead to overlapping phenotypes (*SCN5A* overlap syndromes), where clinical characteristics of LQTS, BrS, and other arrhythmia

 ISSN: 2325-887X DOI: 10.22489/CinC.2018.267

syndromes (conduction disease, sinus node dysfunction) coexist in a single family or even in a single patient [6,13]. Sinus node dysfunction (SND) is a relatively common observation in both LQTS3 and BrS1 patients [14,15].

In a recent study, we investigated the mechanisms of LQTS3-related SND at the cellular level with the use of the comprehensive computational model of a single human SAN cell of Fabbri et al. [5], focusing on the 1795insD gain-of-function mutation in *SCN5A* [16]. In the present study, we assessed the cellular mechanism by which the E161K loss-of-function mutation in *SCN5A* causes BrS-related SND. Also, the potential role of the H558R polymorphism in *SCN5A* was investigated. This highly common polymorphism alters the effects of several mutations in *SCN5A*, including E161K, on sodium channel expression and/or kinetics [17].

1.2. Data from literature

In 2005, Smits et al. [18] reported the biophysical features of a novel sodium channel mutation, E161K, which was identified in individuals of two non-related families with symptoms of sinus bradycardia, sinus node dysfunction, generalized conduction disease, and Brugada syndrome, or combinations thereof. E161K or wild-type sodium channel α-subunit was transfected into tsA201 cells together with wild-type β1-subunit. Voltage clamp experiments on the transfected cells revealed a 2.5-fold reduction in peak I_{Na} at −20 mV for E161K sodium channels compared to wild-type channels. Furthermore, E161K sodium channels showed an 11.9 mV shift of the half-maximal activation potential ($V_{1/2}$) of the steady-state activation curve compared to wild-type (−30.7 ± 0.8 versus −42.6 ± 1.4 mV, respectively). Also, the steepness of this curve was slightly reduced; its slope factor amounted to 7.9 ± 0.3 versus 6.7 ± 0.4 mV, respectively. Voltage dependence of steady-state inactivation, recovery from inactivation, and development of slow inactivation were not found to be affected by the E161K mutation. Of note, these data were obtained in H558 background.

More recently, Gui et al. [17,19] carried out voltage experiments on HEK-293 cells transfected with E161K mutant or wild-type sodium channel α-subunit. Like Smits et al. [18], they observed a reduction in peak I_{Na} for E161K sodium channels compared to wild-type channels, both in H558 and R558 background. In either case, this reduction was approximately 4-fold, which is more pronounced than the 2.5-fold reduction in the study by Smits et al. [18]. With a value of ≈19 mV, in H558 background, the positive shift in $V_{1/2}$ of the steady-state activation curve was also more pronounced. The slope factor of this curve showed an increase similar to that observed by Smits et al. [18].

In R558 background, the reduction in peak I_{Na} and steepness of the steady-state activation curve were both similar to those in H558 background [17]. However, the positive shift in $V_{1/2}$ of the steady-state activation curve was less pronounced, with a value of ≈13 mV rather than ≈19 mV. Furthermore, the steady-state inactivation curve showed a shift of −10 mV in R558 background, whereas such change was absent in H558 background.

2. Methods

Effects of the heterozygous E161K mutation in *SCN5A* were implemented in the CellML code [20] of the Fabbri-Severi human SAN cell model [5] by scaling the fully-activated conductance of I_{Na} and shifting the voltage dependence of I_{Na} activation and/or inactivation, based on the data from literature described in Section 1.2. The slight change in the steepness of the steady-state activation curve was ignored. These modifications were applied to half of the intrinsic I_{Na} channels, thus representing the heterozygous nature of the mutation. Similar changes were applied to the Courtemanche-Ramirez-Nattel human right atrial cell model [21].

In H558 background, the voltage dependence of I_{Na} activation was shifted by +19 mV. In R558 background, the voltage dependence of I_{Na} activation was shifted by +13 mV, together with a −10 mV shift in voltage dependence of inactivation. In either background, a 50% decrease in fully-activated conductance was applied to arrive at a 4-fold decrease in peak current density of I_{Na} during voltage clamp simulations.

The default Fabbri-Severi model has a beating rate of 74 beats/min. This rate was lowered to 49 beats/min through the simulated administration of 20 nM acetyl-choline (ACh; vagal tone) [5]. Simulations of the E161K mutation were carried out both under control conditions (i.e., normal autonomic tone) and under vagal tone.

The CellML code was edited and run in the Cellular Open Resource (COR) environment [22], version 0.9.31.1409. The Courtemanche-Ramirez-Nattel model was coded using Intel Visual Fortran and run on an Intel Core i7 based workstation as a 32-bit Windows application, applying a simple and efficient Euler-type integration scheme with a 1-μs time step. All simulations were run for a sufficiently long time to reach steady-state behavior.

3. Results

We started our simulations with the default SAN cell model (i.e., normal autonomic tone). As shown in Fig. 1, the current through the E161K mutant channels is effectively zero, both in H558 and R558 background. As a result, diastolic depolarization rate decreases and cycle length increases from 813.4 to 867.8 ms. This 54-ms increase in cycle length corresponds with a moderate 6.3% decrease in beating rate from 74 to 69 beats/min.

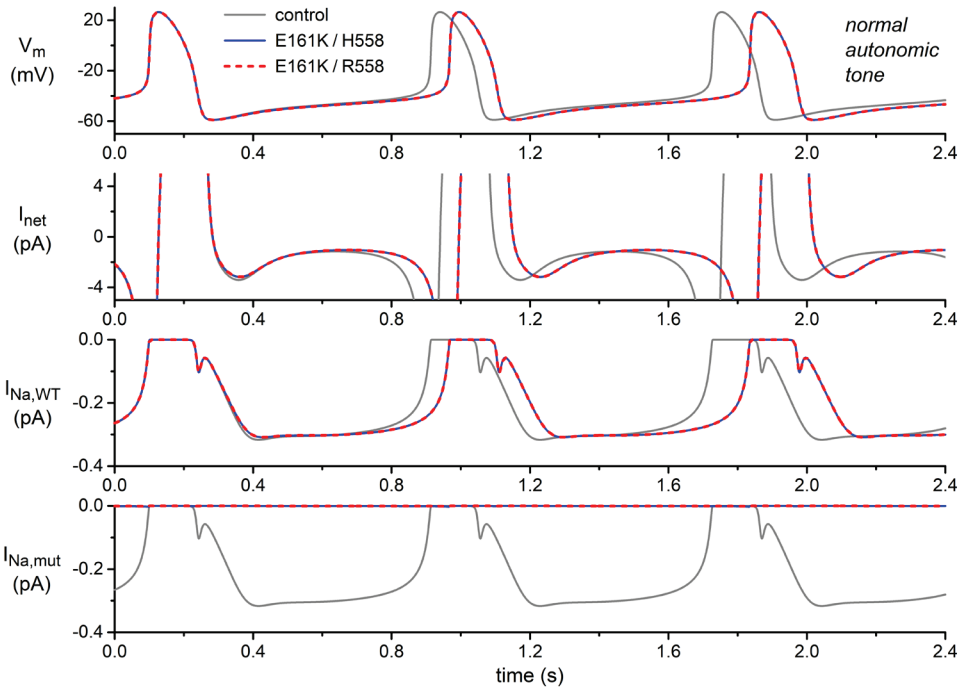

Figure 1. Effect of the heterozygous E161K mutation in *SCN5A* on the electrical activity of the Fabbri et al. [5] human SAN cell model at normal beating rate (normal autonomic tone), both in H558 and R558 background. Membrane potential (V_m), net membrane current (I_{net}), fast sodium current through wild-type channels ($I_{Na,WT}$) and remaining wild-type or mutant channels ($I_{Na,mut}$).

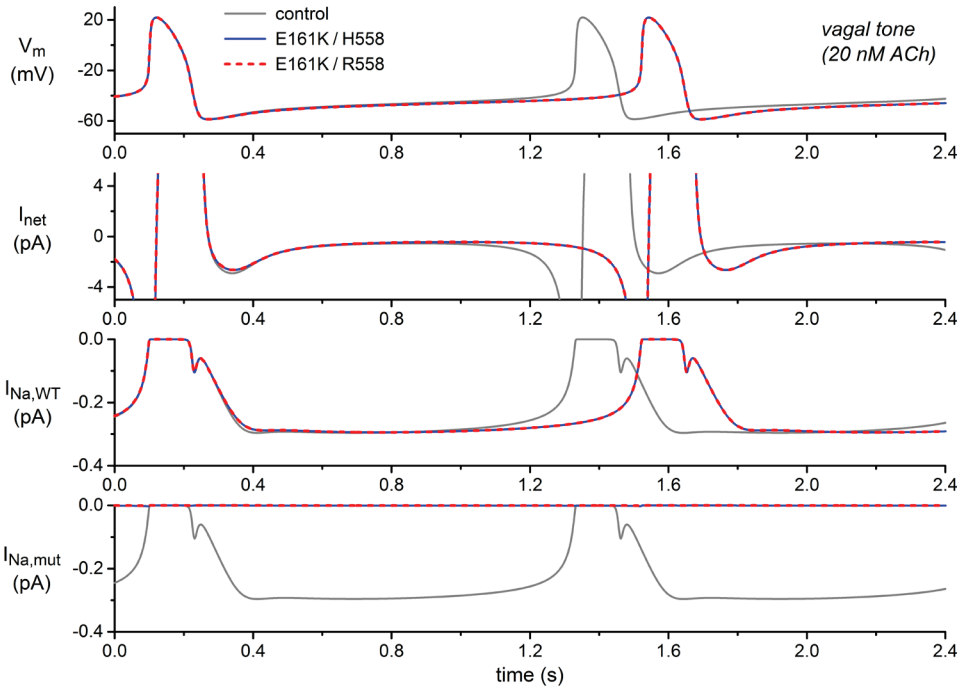

Figure 2. Effect of the heterozygous E161K mutation in *SCN5A* on the electrical activity of the Fabbri et al. [5] human SAN cell model at low beating rate (vagal tone; 20 nM ACh), both in H558 and R558 background. Membrane potential (V_m), net membrane current (I_{net}), fast sodium current through wild-type channels ($I_{Na,WT}$) and remaining wild-type or mutant channels ($I_{Na,mut}$).

We repeated our simulations under vagal tone (20 nM ACh). Vagal tone per se increases cycle length from 813.4 to 1231.3 ms (+51%), which is largely established through a decrease in net membrane current (I_{net}) and associated diastolic depolarization rate. The effect of the E161K mutation is now considerably larger, with a 191-ms increase in cycle length to 1422.6 ms (Fig. 2), corresponding with a 13% decrease in beating rate from 49 to 42 beats/min, in line with the clinical observations by Smits et al. [18].

The E161K mutation does not only increase the intrinsic cycle length of the SAN cells. It also reduces atrial excitability, as demonstrated by the ≈2.2-fold increase in threshold stimulus current of the simulated right atrial cell, virtually independent of the H558 or R558 background (data not shown), adding to the clinically observed sinus bradycardia and sinus node dysfunction.

4. Conclusion

We conclude that the experimentally identified mutation-induced changes in I_{Na} can explain the clinically observed sinus node dysfunction. Furthermore, we conclude that the common H558R polymorphism does not significantly alter the effects of the E161K mutation.

References

[1] Zimmer T, Haufe V, Blechschmidt S. Voltage-gated sodium channels in the mammalian heart. Glob Cardiol Sci Pract 2014;2014:449–63.

[2] Baruscotti M, Barbuti A, Bucchi A. The cardiac pacemaker current. J Mol Cell Cardiol 2010;48:55–64.

[3] DiFrancesco D. The role of the funny current in pacemaker activity. Circ Res 2010;106:434–46.

[4] Verkerk AO, Wilders R, van Borren MMGJ, Tan HL. Is sodium current present in human sinoatrial node cells? Int J Biol Sci 2009;5:201–4.

[5] Fabbri A, Fantini M, Wilders R, Severi S. Computational analysis of the human sinus node action potential: model development and effects of mutations. J Physiol 2017; 595:2365–96.

[6] Giudicessi JR, Wilde AAM, Ackerman MJ. The genetic architecture of long QT syndrome: a critical reappraisal. Trends Cardiovasc Med 2018;28:453–64.

[7] Wilde AAM, Amin AS. Clinical spectrum of *SCN5A* mutations: long QT syndrome, Brugada syndrome, and cardiomyopathy. JACC Clin Electrophysiol 2018;4:569–79.

[8] Brugada J, Campuzano O, Arbelo E, Sarquella-Brugada G, Brugada R. Present status of Brugada Syndrome: *JACC* State-of-the-Art Review. J Am Coll Cardiol 2018;72:1046–59.

[9] Schwartz PJ, Stramba-Badiale M, Crotti L, Pedrazzini M, Besana A, Bosi G, Gabbarini F, Goulene K, Insolia R, Mannarino S, Mosca F, Nespoli L, Rimini A, Rosati E, Salice P, Spazzolini C. Prevalence of the congenital long-QT syndrome. Circulation 2009;120:1761–7.

[10] Hocini M, Pison L, Proclemer A, Larsen TB, Madrid A, Blomström-Lundqvist C. Diagnosis and management of patients with inherited arrhythmia syndromes in Europe: results of the European Heart Rhythm Association Survey. Europace 2014;20:600–3.

[11] Crotti L, Celano G, Dagradi F, Schwartz PJ. Congenital long QT syndrome. Orphanet J Rare Dis 2008;3:18.

[12] Obeyesekere MN, Antzelevitch C, Krahn AD. Management of ventricular arrhythmias in suspected channelopathies. Circ Arrhythm Electrophysiol 2015;8:221–31.

[13] Remme CA, Wilde AAM, Bezzina CR. Cardiac sodium channel overlap syndromes: different faces of *SCN5A* mutations. Trends Cardiovasc Med 2008;18:78–87.

[14] Wilders R, Verkerk AO. Long QT syndrome and sinus bradycardia—a mini review. Front Cardiovasc Med 2018; 5:106.

[15] Hayashi H, Sumiyoshi M, Nakazato Y, Daida H. Brugada syndrome and sinus node dysfunction. J Arrhythm 2018; 4:216–21.

[16] Wilders R. Sinus bradycardia in carriers of the *SCN5A*-1795insD mutation: unraveling the mechanism through computer simulations. Int J Mol Sci 2018;19:2.

[17] Gui J, Wang T, Trump D, Zimmer T, Lei M. Mutation-specific effects of polymorphism H558R in *SCN5A*-related sick sinus syndrome. J Cardiovasc Electrophysiol 2010; 21:564–73.

[18] Smits JPP, Koopmann TT, Wilders R, Veldkamp MW, Opthof T, Bhuiyan ZA, Mannens MMAM, Balser JR, Tan HL, Bezzina CR, Wilde AAM. A mutation in the human cardiac sodium channel (E161K) contributes to sick sinus syndrome, conduction disease and Brugada syndrome in two families. J Mol Cell Cardiol 2005;38:969–81.

[19] Gui J, Wang T, Jones RPO, Trump D, Zimmer T, Lei M. Multiple loss-of-function mechanisms contribute to *SCN5A*-related familial sick sinus syndrome. PLoS One 2010;5:e10985.

[20] Cuellar AA, Lloyd CM, Nielsen PF, Bullivant DP, Nickerson DP, Hunter PJ. An overview of CellML 1.1, a biological model description language. Simulation 2003; 79:740–7.

[21] Courtemanche M, Ramirez RJ, Nattel S. Ionic mechanisms underlying human atrial action potential properties: insights from a mathematical model. Am J Physiol 1998;275:H301–21.

[22] Garny A, Kohl P, Noble D. Cellular Open Resource (COR): a public CellML based environment for modelling biological function. Int J Bifurcat Chaos 2003;13:3579–90.

Address for correspondence:

Ronald Wilders, PhD
Department of Medical Biology
Academic Medical Center, University of Amsterdam
Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands
Phone: +31-20-5665229
E-mail: r.wilders@amc.uva.nl