Mechanism of Sinus Bradycardia in Carriers of the 1795insD Mutation in the *SCN5A* Gene

Ronald Wilders

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

Abstract

The SCN5A gene encodes the pore-forming α-subunit of the cardiac fast sodium channel (I_{Na} channel). Carriers of the 1795insD mutation in SCN5A show sinus bradycardia, with a mean heart rate of 70 bpm in mutation carriers vs. 77 bpm in non-carriers from the same family (lowest heart rate 41 vs. 47 bpm).

We assessed the mechanism by which the 1795insD mutation causes sinus bradycardia by incorporating the mutation-induced changes in I_{Na} into the comprehensive computational model of a single human sinoatrial node cell that was recently developed by Fabbri et al.

The 1795insD mutation reduced the beating rate of the model cell from 74 to 69 bpm (from 49 to 43 bpm in the presence of 20 nM acetylcholine). The mutation-induced persistent I_{Na} per se resulted in a large increase in beating rate. This gain-of-function effect was almost completely counteracted by the loss-of-function effect of the reduction in I_{Na} amplitude. The further loss-of-function effect of the shifts in (in)activation resulted in an overall loss-of-function effect of the 1795insD mutation.

We conclude that the experimentally identified mutation-induced changes in I_{Na} can explain the clinically observed sinus bradycardia. Furthermore, we conclude that the Fabbri et al. model may prove a useful tool in understanding cardiac pacemaker activity in human.

1. Introduction

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), which shows an estimated prevalence of 1:2,000 [6] and is the most commonly encountered inherited arrhythmia disorder in clinical practice (≈35% [7]). 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 prolongation of the long QT interval on the ECG. The estimated prevalence of LQT3 among LQTS patients is ≈10% [8,9].

An intriguing and widely studied mutation among the mutations in *SCN5A* associated with LQT3 is 1795insD, which is not only characterized by QT prolongation, but also by atrial and ventricular conduction delays, and nocturnal sudden cardiac death. Carriers of this mutation may not only present with LQT3, but also with Brugada syndrome and with sinus bradycardia [10]. In line with the conduction delays and the Brugada phenotype, loss-of-function mutational effects were observed in patch clamp experiments on wild-type and mutant Na^+ channels expressed in *Xenopus* oocytes. The steady-state activation curve was shifted by +8.1 mV, whereas the steady-state inactivation curve was shifted by −7.3 mV. Also, the fully-activated I_{Na} was reduced by 78% [10]. The QT prolonging effects could be explained by the persistent I_{Na} of ≈1.5% (percent of peak I_{Na}) that was observed in patch clamp experiments on wild-type and mutant Na^+ channels expressed in HEK-293 cells [11].

Holter recording in 1795insD patients revealed sinus bradycardia with an ≈11% decrease in minimum, average,

 ISSN: 2325-887X DOI:10.22489/CinC.2017.021-424

Table 1. Clinical observations on heart rate in carriers of mutations in *SCN5A* or *HCN4*.

	HR in mutation carriers (bpm)				HR in non-carriers (bpm)				
Mutation	Min	Avg	Max	*n*	Min	Avg	Max	*n*	Study
In SCN5A									
1795insD	41 ± 1**	70 ± 1**	124 ± 3**	54	47 ± 1	77 ± 2	141 ± 3	40	Van den Berg et al. [12]
In HCN4									
G480R	32 ± 8*	49 ± 12*	101 ± 21*	7	55 ± 9	73 ± 11	126 ± 17	8	Nof et al. [13]
A485V	37 ± 3*	58 ± 6*	117 ± 27	14	49 ± 11	77 ± 12	140 ± 33	5	Laish-Farkash et al. [14]
695X	36 ± 6*	56 ± 5*	131 ± 17	7	47 ± 6	72 ± 10	157 ± 26	6	Schweizer et al. [15]

Minimum (Min), average (Avg), and maximum (Max) heart rate (HR) obtained with 24-hour Holter recording. Data are mean ± SEM. *P<0.05 vs. non-affected family members. **P<0.001 vs. non-affected family members.

and maximum heart rate [12], as detailed in Table 1, which also shows the bradycardic effect of some typical *HCN4* mutations [13–15] for comparison.

We studied the mechanisms of the 1795insD mutation-induced bradycardia at the cellular level with the use of the comprehensive computational model of a single human SAN cell of Fabbri et al. [5].

2. Methods

Effects of the heterozygous 1795insD mutation in *SCN5A* were implemented in the CellML code [16] of the Fabbri et al. human SAN cell model [5] by scaling the fully-activated conductance of I_{Na}, shifting the voltage dependence of I_{Na} activation and inactivation, and introducing a percentage of non-inactivating I_{Na} channels, based on the data from literature described in the Introduction. These modifications were applied to half of the intrinsic I_{Na}, thus representing the heterozygous nature of the mutation.

The default Fabbri et al. model [5] has a beating rate of 74 bpm. This rate was lowered to 49 bpm (vagal tone) through the simulated administration of 20 nM acetyl-choline (ACh). A beating rate of 140 bpm (β-adrenergic tone) was obtained through the simulated administration of isoprenaline (Iso), tuning the parameters affected by Iso to arrive at this beating rate.

The CellML code was edited and run in the Cellular Open Resource (COR) environment [17], version 0.9.31.1409. All simulations were run for a sufficiently long time to reach steady-state behaviour.

3. Results

We started our simulations with the default SAN cell model (normal autonomic tone). The gain-of-function effect of the 1795insD mutation, i.e., the 1.5% persistent sodium current, shortens the cycle length of the model cell from 813 to 683 ms through a moderate increase in action potential duration (Fig. 1A) and a more substantial increase in diastolic depolarization rate (Fig. 1A, red and dashed grey traces). The latter is caused by an increase in the net inward current during diastolic depolarization as a result of the increased I_{Na} (Fig. 1, B and C).

The gain-of-function effect of the persistent current is completely counteracted by the loss-of-function effect of the 78% decrease in the fully-activated I_{Na} conductance (Fig. 1, green traces). With the additional loss-of-function effect of the shifts in voltage dependence of I_{Na} activation and inactivation, the net effect of the 1795insD mutation is an inhibition of I_{Na} that results in an increase in cycle length from 813 to 867 ms (Fig. 1, blue traces).

As illustrated in Fig. 2, the effects of the 1795insD mutation are highly similar in the presence of 20 nM ACh (vagal tone). Similar mutation effects (data not shown) were observed at high beating rate through the simulated administration of isoprenaline (β-adrenergic tone).

The mutation effects on beating rate observed in our computer simulations are summarized in Fig. 3, together with the clinical observations on the heart rate of heterozygous 1795insD mutation carriers by Van den Berg et al. [12]. The simulation results match the clinical data, but the decrease in beating rate of ≈8% is somewhat smaller than the clinically observed decrease in heart rate of ≈11%. In our single cell simulations, there is no hyperpolarizing effect on the SAN cell of the surrounding atrial tissue. Such hyperpolarizing effect may increase the availability of sodium channels and thus the effect of the (mutated) sodium current on beating rate.

4. Conclusion

We conclude that the experimentally identified effects of the 1795insD mutation on I_{Na} can explain the clinically observed sinus bradycardia. Together, the loss-of-function effects of the shifts in the voltage dependence of

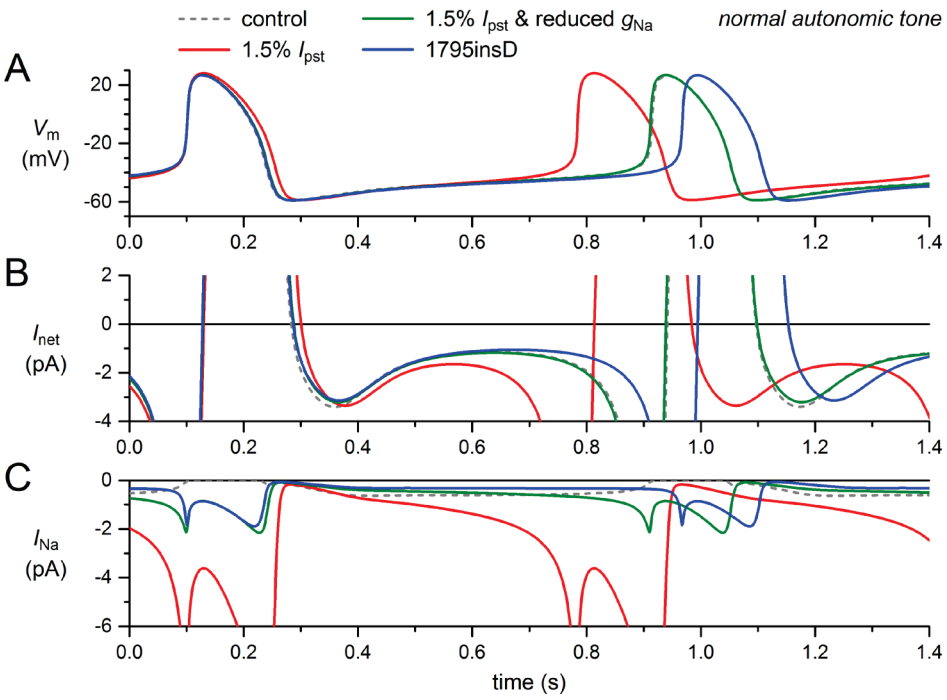

Figure 1. Effect of the heterozygous 1795insD mutation in *SCN5A* on the electrical activity of the Fabbri et al. [5] human SAN cell model at normal beating rate (normal autonomic tone). (A) Membrane potential (V_m). (B) Net membrane current (I_{net}). (C) Fast sodium current (I_{Na}). Net effect of the mutation (blue traces), effect of the persistent I_{Na} per se (red traces), and effect of the persistent I_{Na} in combination with a reduction in I_{Na} conductance (green traces).

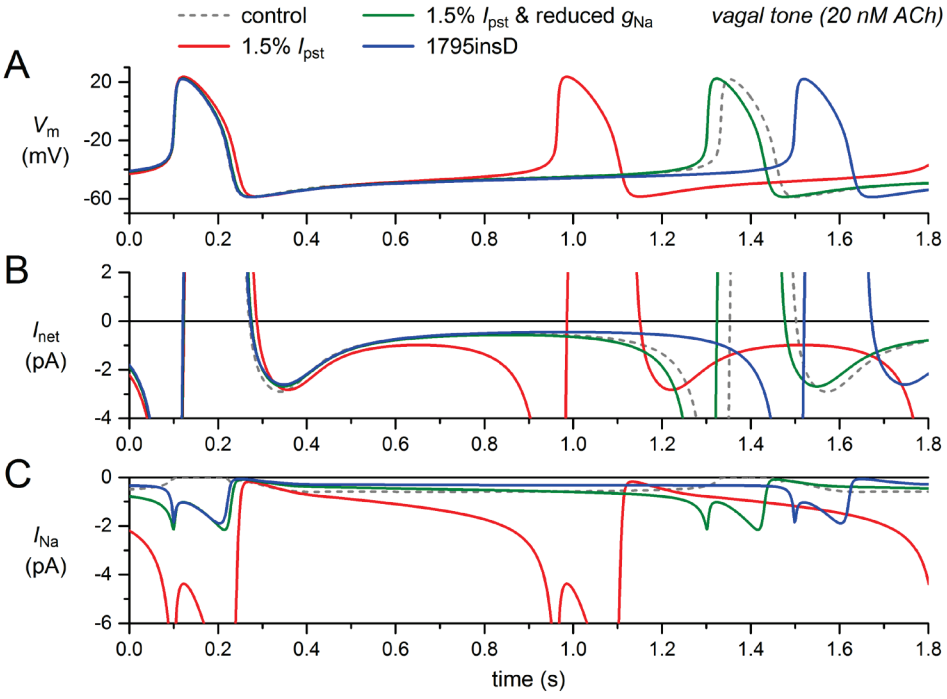

Figure 2. Effect of the heterozygous 1795insD mutation in *SCN5A* on the electrical activity of the Fabbri et al. [5] human SAN cell model at low beating rate (vagal tone; 20 nM ACh). (A) Membrane potential (V_m). (B) Net membrane current (I_{net}). (C) Fast sodium current (I_{Na}). Net effect of the mutation (blue traces), effect of the persistent I_{Na} per se (red traces), and effect of the persistent I_{Na} in combination with a reduction in I_{Na} conductance (green traces).

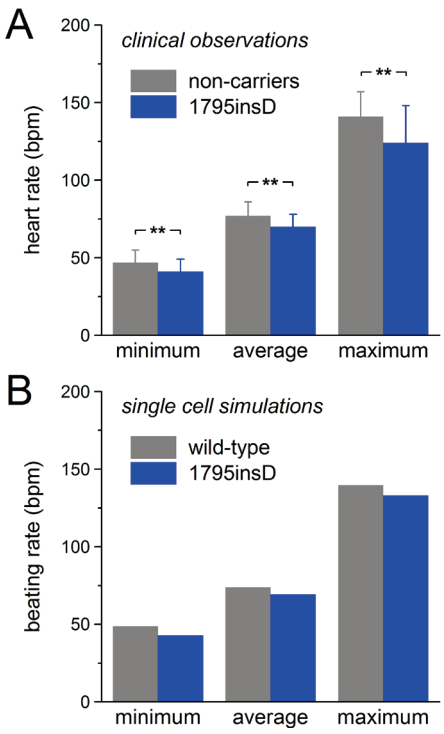

Figure 3. Effect of the heterozygous 1795insD mutation in *SCN5A*. (A) Clinical observations on heart rate. Data are mean ± SD obtained from Holter recordings (54 mutation carriers, 40 non-carriers; $^{**}P<0.001$) [12]. (B) Beating rate of Fabbri et al. [5] human SAN cell model.

I_{Na} activation and inactivation and the decrease in fully-activated conductance are stronger than the gain-of-function effect of the persistent current, resulting in an overall increase in cycle length through a decrease in net inward current during the final phase of diastolic depolarization. Furthermore, we conclude that the Fabbri et al. model may prove a useful tool in understanding cardiac pacemaker activity in human.

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] 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.

[7] 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;16:600–3.

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

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

[10] Bezzina C, Veldkamp MW, van den Berg MP, Postma AV, Rook MB, Viersma JW, van Langen IM, Tan-Sindhunata G, Bink-Boelkens MTE, van der Hout AH, Mannens MMAM, Wilde AAM. A single Na^+ channel mutation causing both long-QT and Brugada syndromes. Circ Res 1999;85:1206–13.

[11] Veldkamp MW, Wilders R, Baartscheer A, Zegers JG, Bezzina CR, Wilde AAM. Contribution of sodium channel mutations to bradycardia and sinus node dysfunction in LQT3 families. Circ Res 2003;92:976–83.

[12] van den Berg MP, Wilde AAM, Viersma JW, Brouwer J, Haaksma J, van der Hout AH, Stolte-Dijkstra I, Bezzina CR, Van Langen IM, Beaufort-Krol GCM, Cornel JH, Crijns HJGM. Possible bradycardic mode of death and successful pacemaker treatment in a large family with features of long QT syndrome type 3 and Brugada syndrome. J Cardiovasc Electrophysiol 2001;12:630–6.

[13] Nof E, Luria D, Brass D, Marek D, Lahat H, Reznik-Wolf H, Pras E, Dascal N, Eldar M, Glikson M. Point mutation in the HCN4 cardiac ion channel pore affecting synthesis, trafficking, and functional expression is associated with familial asymptomatic sinus bradycardia. Circulation 2007;116:463–70.

[14] Laish-Farkash A, Glikson M, Brass D, Marek-Yagel D, Pras E, Dascal N, Antzelevitch C, Nof E, Reznik H, Eldar M, Luria D. A novel mutation in the *HCN4* gene causes symptomatic sinus bradycardia in Moroccan Jews. J Cardiovasc Electrophysiol 2010;21:1365–72.

[15] Schweizer PA, Duhme N, Thomas D, Becker R, Zehelein J, Draguhn A, Bruehl C, Katus HA, Koenen M. cAMP sensitivity of HCN pacemaker channels determines basal heart rate but is not critical for autonomic rate control. Circ Arrhythm Electrophysiol 2010;3:542–52.

[16] 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.

[17] 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