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Biophysical phenotypes of *SCN5A* mutations causing long QT and Brugada syndromes

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Abstract Long QT and Brugada syndromes are two hereditary cardiac diseases. Brugada syndrome has so far been associated with only one gene, *SCN5A*, which encodes the cardiac sodium channel. However, in long QT syndrome (LQTS) at least six genes, including the *SCN5A*, are implicated. The substitution (D1790G) causes LQTS and the insertion (D1795) induces both LQTS and Brugada syndromes in carrier patients. hH1/insD1795 and hH1/D1790G mutant channels were expressed in the tsA201 human cell line and characterized using the patch clamp technique in whole-cell configuration. Our data revealed a persistent inward sodium current of about 6% at −30 mV for both D1790G and insD1795, and a reduction of 62% of channel expression for the insD1795. Moreover, a shift of steady-state inactivation curve in both mutants was also observed. Our findings uphold the idea that LQT3 is related to a persistent sodium current whereas reduction in the expression level of cardiac sodium channels is one of the biophysical characteristics of Brugada syndrome.

Key words: Brugada syndrome; Sodium channel; *SCN5A*; Long QT syndrome; Ventricular fibrillation; Arrhythmia

1. Introduction

Long QT syndrome (LQTS) and Brugada syndrome are two inherited cardiac disorders that cause ventricular fibrillation and sudden death. Clinically, LQTS is characterized by an abnormal QT interval prolongation in 12 leads electrocardiogram (ECG) [1–3]. However, Brugada syndrome is characterized by an ST segment elevation in the right leads (V1, V2 and V3) of the ECG, a pseudo right bundle branch block and a normal QT interval [4,5]. No structural abnormalities have been identified in patients with either LQTS or Brugada syndrome. Mutations in six genes encoding different ionic channels or their accessory subunits were identified in LQTS patients. However, Brugada syndrome mutations have so far been identified in only one gene, *SCN5A*, the cardiac sodium channel gene [6–8].

Although LQTS and Brugada syndrome are known to be distinct disorders, a recent study reported a novel mutation in *SCN5A* gene that causes both syndromes [8]. In their report [8], an in frame insertion of three nucleotides in the C-terminus region of cardiac sodium channel resulting in an incorporation of aspartate at position 1795 was identified as being responsible for both LQTS and Brugada syndrome phenotype in one family. In their report, the authors correlated the Brugada syndrome phenotype with a decrease in sodium channel expression in *Xenopus* oocytes. However, the authors did not find any residual current, which represents the principal biophysical phenotype of mutations causing LQTS in *SCN5A* known as LQT3 [9]. The reason as to why no residual current could be recorded is not clear. Recent biophysical data revealed a residual current when this mutation was expressed in the HEK293 cell line [10]. This study reported that the insertion mutant also affects slow inactivation and therefore contributes to Brugada syndrome features. Other studies have reported that the acceleration of the current decay may also cause Brugada syndrome phenotypes [11,12].

On the other hand, the physiopathology of D1790G mutation found in a patient with LQTS was shown to be related to α–β_1 subunit interaction [13]. The data presented were not clear as to how this could involve QT prolongation. However, subsequent study from the same authors described that D1790G mutation carriers responded to flecainide treatment but not to lidocaine by rectifying QTc interval [14]. The occurrence of a residual current due to the reduction of sodium channel inactivation would increase the action potential duration leading to early after depolarizations, QT prolongation, which would then degenerate into ventricular fibrillation.

In this study, we expressed hH1/insD1795 and hH1/D1790G channels in tsA201 cells (human embryonic kidney cells) [15]. Our data revealed a reduction in sodium channel expression and the presence of a persistent inward sodium current in the case of insD1795, thus explaining both clinical phenotypes in patients carrying this mutation. A persistent inward sodium current could explain the LQTS features in patients carrying the hH1/D1790G variant of the cardiac sodium channel.

2. Materials and methods

2.1. Mutagenesis

Mutants hH1/insD1795 and hH1/D1790G were generated using QuickChange TM site-directed mutagenesis kit, according to the manufacturer's instructions (Stratagene, La Jolla, CA, USA).

Mutants were constructed using the following mutagenic sense and antisense primers, respectively:

For the insertion D1795: 5′-CTT CGA TAT GTT CTA TGA TGA GAT CTG GGA GAA ATT TG-3′ and 5′-CA AAT TTC TCC CAG ATC TCA TCA TAG AAC ATA TCG AAG-3′.

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For the mutant D1790G: 5′-GAG CCC CTG AGT GAG GAC GGC TTC GAT ATG TTC TAT GAG-3′ and 5′-CTC ATA GAA CAT ATC GAA GCC GTC CTC ACT CAG GGG CTC-3′.

Underlined are the inserted nucleotides.

Mutant and the wild-type hH1 in a pcDNA1 construct were purified using Quiagen columns (Quiagen Inc., Chatsworth, CA, USA).

2.2. Transfections of the tsA201 cell line

The mammalian cell line tsA201 is derived from human embryonic kidney HEK293 cells by stable transfection with SV40 large T antigen. Cells were grown in high glucose DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% fetal bovine serum, L-glutamine (2 mM), penicillin G (100 U/ml) and streptomycin (10 mg/ml) (Gibco BRL Life technologies, Burlington, Ont., Canada). Cells were incubated in a 5% CO_2 humidified atmosphere. The tsA201 cells were transfected using the calcium phosphate method, with the following modification: to better identify individual transfected cells, 10 μl of piERS/CD8/β plasmid was cotransfected with 10 μl of either the wild-type or the mutant hH1 sodium channels cDNA. The human sodium channel β-subunit and CD8 were constructed in piERS vector (piERS/CD8/β). Using this strategy, transfected cells that bind beads will also express the β-subunit. For patch clamp experiments, 2–3 days post-transfection cells were incubated for 2 min in a medium containing anti-CD8-a coated beads (Dynabeads M-450 CD8-a) [16]. The unattached beads were removed by washing with extracellular solution. Beads were prepared according to the manufacturer's instructions (Dynal A.S., Oslo, Norway). Cells expressing CD8-a on their surface fixed the beads and were distinguished from non-transfected cells by light microscopy.

2.3. Patch clamp method

Macroscopic sodium currents from transfected cells were recorded using the whole-cell configuration of the patch clamp technique. Patch electrodes were made from 8161 Corning glass and coated with Sylgard (Dow-Corning, Midland, MI, USA) to minimize their capacitance. Low resistance electrodes (<0.8 MΩ) were used, and a routine series resistance compensation of an Axopatch 200 amplifier was performed to values $>80\%$ to minimize voltage-clamp errors. Voltage-clamp command pulses were generated by microcomputer using pCLAMP software v5.5 (Axon Instruments, Foster City, CA, USA). Recorded sodium currents were filtered at 5 kHz. Experiments were performed 10 min after obtaining the whole-cell configuration to allow the current to stabilize.

2.4. Solutions and reagents

For whole-cell recording, the patch pipet contained 35 mM NaCl, 105 mM CsF, 10 mM EGTA, and 10 mM Cs-HEPES (pH = 7.4). The extracellular solution contained 150 mM NaCl, 2 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, and 10 mM Na-HEPES (pH = 7.4). A correction of the liquid junction potential of −7 mV between patch pipet and the bath solutions was made. Experiments were performed at ambient temperature (22–23°C).

2.5. Statistical analysis

Data are expressed as mean ± S.E.M.

When indicated, *t*-test was performed using statistical software in SigmaPlot (Jandel Scientific Software, San Rafael, CA, USA). Differences were deemed significant at a P value <0.05.

3. Results

Macroscopic sodium currents from wild-type (hH1/WT), and hH1 mutants (hH1/D1790G and hH1/insD1795) coexpressed with the β-subunit were recorded in whole-cell configuration from transfected tsA201 cells. Representative inward and outward current traces from hH1/WT, hH1/D1790G and hH1/insD1795 transfected cells are shown in Fig. 1. Wild-type and mutant D1790G sodium currents were characterized by a large amplitude and fast activation and inactivation kinetics

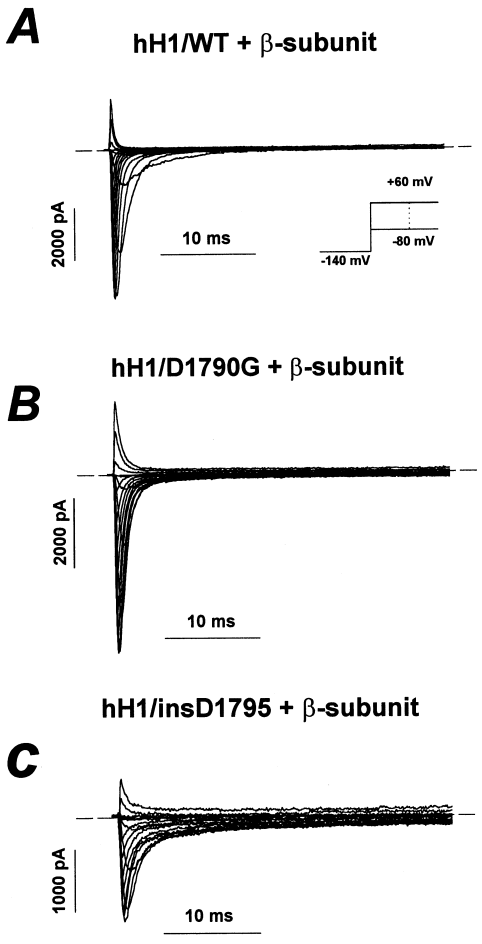

Fig. 1. Whole-cell sodium current traces from hH1/WT+β-subunit (A), hH1/D1790G+β-subunit (B) and hH1/insD1795+β-subunit (C) expressed in tsA201 cell line. Currents were generated from a holding potential of −140 mV from −80 to +60 mV during 30 ms in 10 mV increments. The dashed line represents the zero current.

(Fig. 1A,B). In contrast, hH1/insD1795 sodium currents were characterized by smaller amplitudes (Fig. 1C).

Although the same amount of DNA (10 μg) was used for transfection with both WT and insD1795, the recorded sodium currents for insD1795 were significantly smaller than for WT channels. Fig. 3A illustrates this fact, current/voltage relationship (Fig. 3A) for both hH1/WT and hH1/insD1795 shows that hH1/insD1795 resulted in 62% ($P<0.05$) reduction of sodium currents density. Sodium current densities were corrected for membrane capacitance of each cell.

Both mutants hH1/D1790G and hH1/insD1975 showed persistent inward sodium currents of about 6% at −30 mV (Fig. 1B,C). The persistent sodium current is due to late sodium channels activity; this was demonstrated by using tetrodotoxin (TTX), a specific sodium channel blocker (Fig. 2A,B). TTX at 10 μM reduced the persistent inward sodium current in both hH1/D1790D and hH1/insD1795 to almost zero.

In both hH1/D1790G and hH1/insD1795, steady-state activation curve was shifted to about 10 mV and 5 mV, respectively, toward the more positive voltages (Figs. 3B and 4B). The $V_{1/2}$ values are -38.10 ± 2.30 mV for hH1/insD1795+β ($n=9$) and -41.88 ± 1.82 mV for hH1/D1790G ($n=9$) versus -47.24 ± 1.70 mV for the hH1/WT+β ($n=23$); ($P<0.05$).

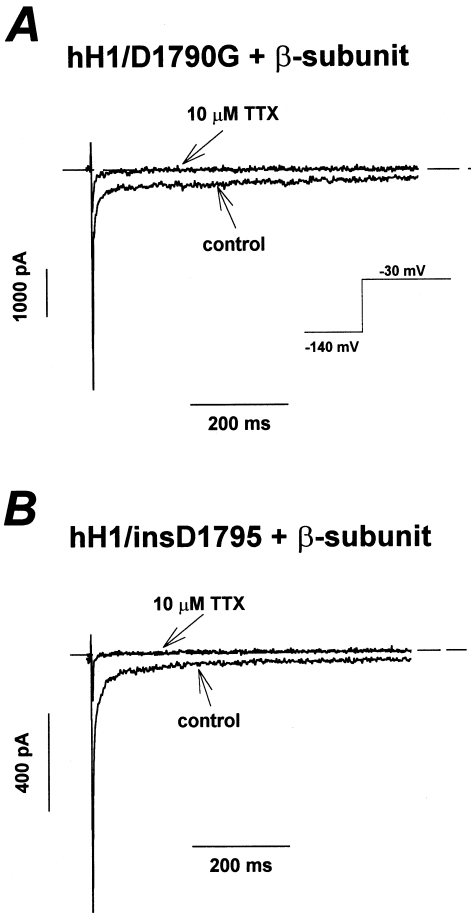

Fig. 2. (A) Effect of 10 μM of TTX on sodium current recorded from cells expressing hH1/D1790G. (B) Effect of 10 μM of TTX on sodium current recorded from cells expressing hH1/insD1795. Sodium currents from both hH1/insD1795+β and hH1/D1790G were recorded from a holding potential of −140 mV to a voltage test of −30 mV, before and at steady-state TTX effect. The dashed line represents the zero current.

The slope factor for steady-state activation was significantly affected in mutated channels ($P < 0.05$), -13.10 ± 1.20 mV ($n = 9$) for hH1/insD1795 and -10.04 ± 0.59 mV ($n = 8$) for hH1/D1790G versus -6.56 ± 0.37 mV for the wild-type ($n = 21$). Steady-state inactivation was also studied using a two pulse protocol with 500 ms pre-pulse duration and the averaged data were fitted with a Boltzmann function. The steady-state inactivation curve in mutants D1790G and insD1795 was shifted by nearly 20 mV toward more hyperpolarized voltages (Figs. 3B and 4B). The $V_{1/2}$ values for steady-state inactivation are -107.86 ± 1.86 mV for hH1/D1790G+β ($n = 9$) and -112.4 ± 2.73 mV for hH1/insD1795+β ($n = 20$) versus -93.22 ± 1.13 mV for hH1/WT+β ($n = 21$); ($P < 0.05$). The slope factor for steady-state inactivation curves was not significantly affected in both mutant channels.

4. Discussion

All reported mutations on the *SCN5A* gene inducing LQTS, known as LQT3, induce a persistent sodium current and lead to QT interval prolongation [17–19] with the exception of the D1790G mutant which was reported to disrupt α–β interaction [13]. Our study revealed a residual sodium current in the presence of each of these mutants, hH1/D1790G and hH1/insD1795. It is not clear why An et al. did not record any residual current [13]. However, there are few explanations to these differences; importantly, in contrast to their study we used 35 mM sodium in the patch pipet. We employed cesium fluoride instead of cesium aspartate to block potassium channels. There are other technical aspects that were not addressed in their study, for example, it is not clear at what time after breaking the seal the recordings were performed, because of the shift of gating that occurs over time while recording sodium currents [20]. We cannot however speculate on the basis of the precise differences.

In the original study of the insertion mutation (insD1795) expressed in *Xenopus* oocytes [8] no residual current was recorded. However, a subsequent study of insD1795 reported the presence of residual current when this mutation was expressed in the HEK293 cells [10].

Our data support the idea that the aspartate insertion at position 1795 could induce both the LQTS and the Brugada syndrome. The presence of a residual current may prolong the myocardial action potential duration and hence the QT interval on the ECGs of patients carrying the insD1795 or the D1790G. In addition, the 62% reduction of sodium channel

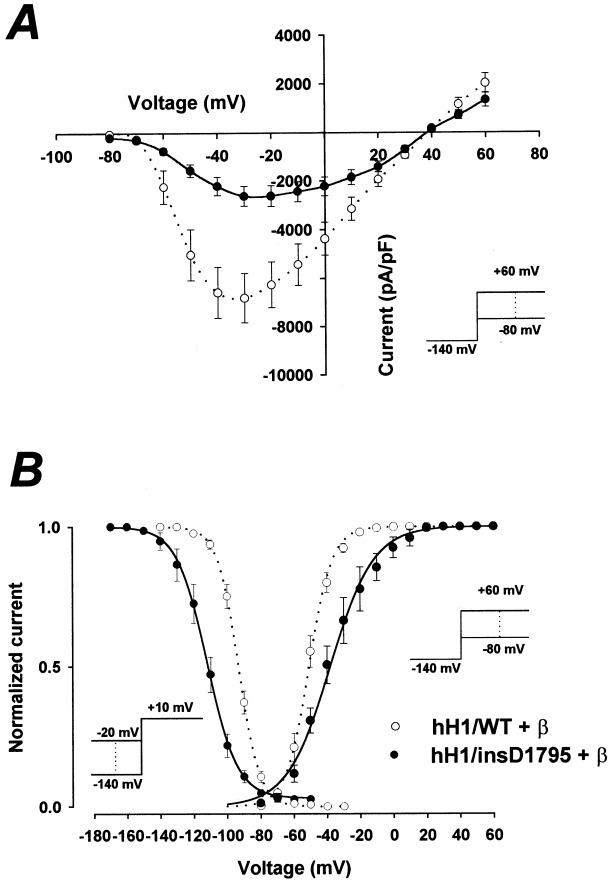

Fig. 3. Current–voltage relationship for normalized amplitudes of sodium currents to the cell capacitance of both hH1/WT+β ($n = 22$) with dotted line (open circles) and hH1/insD1795+β ($n = 21$) with straight line (black circles) (A). Steady-state voltage dependence of activation and inactivation curves in normal and mutated sodium channels fitted to a Boltzmann distribution (B).

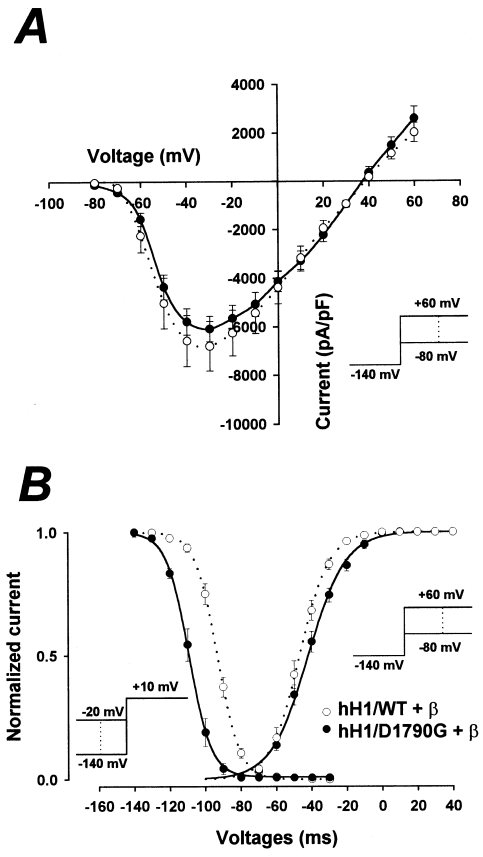

Fig. 4. Current–voltage relationship for normalized amplitudes of sodium currents to the cell capacitance of both hH1/WT+β ($n=22$) with dotted line (open circles) and hH1/D1790G+β ($n=9$) with straight line (black circles) (A). Steady-state Voltage dependence of activation and inactivation curves in normal and mutated sodium channels fitted to a Boltzmann distribution (B).

expression could well be the main cause of Brugada syndrome phenotype in patients carrying the insD1795 mutation. It was also reported that this insertion mutant could also interfere with recovery from intermediate inactivation, this would contribute to Brugada phenotype [10]. This reduction in sodium currents is unlikely to be related to the slowing of recovery from the intermediate state of inactivation which has a time constant of 92.7 ms [10]. In our study we used a 0.2 Hz pulse frequency (5 s interpulse duration) for recording sodium currents. Furthermore, longer interpulse durations (10, 20 and 30 s) were also studied and only slight effects were observed (data not shown). Moreover, recovery from fast inactivation at holding potentials −130 mV and −140 mV of hH1/D1790G, hH1/insD1795 and hH1/WT did not differ (data not shown). Sodium currents are further diminished due to the 20 mV shift of the steady-state inactivation curve and this could contribute to the Brugada phenotype.

Most of the mutations causing the Brugada syndrome result in either a reduction of sodium channel expression [6,7] or a slowing of recovery from fast inactivation [7,15]. Recently, Dumaine et al. reported that the T1620M mutation in hH1 sodium channels causing the Brugada syndrome accelerates current decay at 37°C [11]. In their report, slowing of recovery from fast inactivation compared to the wild-type was also observed but not considered to be responsible for the Brugada syndrome phenotype. More recently the T1620M mutation was also found to enhance sodium channels intermediate inactivation [21].

It is now well accepted that reduction in sodium currents (smaller depolarizing inward current) favors potassium outward currents (abundant in the epicardium [22]) leading to a heterogeneity of repolarization between endocardial and epicardial layer of the ventricle, seen as an ST segment elevation in the right leads of the ECG. We suspect that the reduction of sodium channel expression will also result in the shortening of action potential duration in both endocardium and epicardium [23]. However, in the epicardium the activation of I_{to} (transient outward current) would further reduce the duration of the action potential and thus inducing ST segment elevation and the Brugada syndrome phenotype.

The insertion of an extra negative charge residue D1795 in the C-terminal region, which is already highly acidic, could disturb an electrostatic interaction with the rest of the channel or even induce a disruptive change in the conformation of the C-terminal region [24].

In conclusion, our data suggest that a single amino acid mutation could have a double consequence on the biophysical properties of the channel resulting in distinct clinical phenotypes. Hence, our data support the concept that LQT3 is related to a gain of cardiac sodium channel function, whereas the Brugada syndrome is associated with a loss of function of these channels.

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