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Impaired Na_v1.2 Function and Reduced Cell Surface Expression in Benign Familial Neonatal-Infantile Seizures

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SUMMARY

Purpose—Mutations in *SCN2A*, the gene encoding the brain voltage-gated sodium channel α -subunit Na_v1.2, are associated with inherited epilepsies including benign familial neonatal-infantile seizures (BFNIS). Functional characterization of three BFNIS mutations was performed to identify defects in channel function that underlie this disease.

Methods—We examined three BFNIS mutations (R1319Q, L1330F, and L1563V) using whole-cell patch-clamp recording of heterologously expressed human Na_v1.2. Membrane biotinylation was employed to examine the cell surface protein expression of the four Na_v1.2 alleles.

Results—R1319Q displayed mixed effects on activation and fast inactivation gating, consistent with a net loss of channel function. L1563V exhibited impaired fast inactivation predicting a net gain of channel function. The L1330F mutation significantly decreased overall channel availability to repetitive stimulation. Patch-clamp analysis also revealed that cells expressing BFNIS mutants exhibited lower levels of sodium current compared to wildtype Na_v1.2 (WT). Biochemical experiments demonstrated that all three BFNIS mutations exhibited a significant reduction in cell surface expression compared to WT.

Discussion—Our findings indicate that BFNIS is associated with a range of biophysical defects accompanied by reduced levels of channel protein at the plasma membrane.

Keywords

Sodium Channel; Inherited Epilepsy; Basic Electrophysiology; Na_v1.2; SCN2A

INTRODUCTION

Voltage-gated sodium channels are responsible for the initiation and propagation of action potentials in excitable tissues. These heteromultimeric complexes are comprised of a large (~260 kDa) pore-forming α subunit and smaller (~30 kDa) β accessory subunits. The α subunit is comprised of four homologous domains (DI-DIV) and exhibits significant homology to voltage-gated potassium and calcium channels. Recent structural studies have shown functional voltage-sensing and central pore subdomains within the predicted four-fold pseudo-symmetrical DI-DIV arrangement (Fig. 1A) (Catterall, 2000). Abnormal

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biophysical activity caused by mutations within channel subdomains is a common theme in inherited channelopathies, and epilepsy in particular.

Mutations in three neuronal voltage-gated sodium channel genes (*SCN1A*, *SCN2A*, and *SCN1B*) have been associated with a genetic predisposition to epilepsy (Harkin et al., 2007; Meisler and Kearney, 2005). Specifically, most mutations in *SCN2A*, encoding the pore-forming subunit of Nav1.2, have been identified in cases of benign familial neonatal-infantile seizures (BFNIS) (Berkovic et al., 2004; Herlenius et al., 2007; Heron et al., 2002) (Fig. 1A). This epileptic syndrome is characterized by the onset of afebrile generalized seizures at an early age and spontaneous remission within the first year of life. The onset of symptoms in BFNIS, typically between 2 days and 3.5 months, overlaps that of benign familial neonatal convulsions (BFNC) and benign familial infantile seizures (BFIS) (Kaplan and Lacey, 1983). BFNC caused by mutations in *KCNQ2* and *KCNQ3* potassium channel genes has an earlier age of onset (Charlier et al., 1998; Singh et al., 1998). BFIS shares some clinical features with BFNIS, but has a later onset (after three months) and can be associated with mutations in *SCN2A*, *ATP1A2*, as well as other as yet unidentified genes (Striano et al., 2006). Seizures associated with BFNIS respond well to anticonvulsants and spontaneously remit within the first year of life without long term neurological sequelae. BFNIS exhibits autosomal dominant inheritance with a high degree of penetrance (Berkovic et al., 2004).

Mutations in Nav1.2 have been less frequently associated with more severe forms of epilepsy. A missense *SCN2A* mutation was identified in a generalized epilepsy with febrile seizures plus (GEFS+) patient (Sugawara et al., 2001) and a truncation mutation was identified in a patient exhibiting features of severe myoclonic epilepsy of infancy (SMEI) (Kamiya et al., 2004). These two mutations appear to cause severe defects in channel activity (Kamiya et al., 2004; Sugawara et al., 2001). However, the relationships among specific mutations, channel dysfunction and corresponding clinical phenotype are not well defined for benign *SCN2A*-associated epilepsies, like BFNIS.

To date, eight *SCN2A* mutations have been associated with BFNIS (Fig. 1A) (Berkovic et al., 2004; Herlenius et al., 2007; Heron et al., 2002). The BFNIS mutations affect different regions of the protein structure. Four of the BFNIS mutations have been studied previously by overexpressing the α subunit alone in either rat cortical neurons or HEK293T cells (Scalmani et al., 2006; Xu et al., 2007). Here we present the functional characterization of three BFNIS mutations (R1319Q, L1330F and L1563V) using a recombinant human Nav1.2 expressed with the accessory subunits, $\text{h}\beta_1$ and $\text{h}\beta_2$, in an effort to better define functional defects that may be unique to BFNIS. We found that these mutations had mixed effects on channel activity predicting either loss (R1319Q, L1330F) or gain (L1563V) of function. Unexpectedly, all three BFNIS mutations exhibited substantially lower levels of protein expression at the cell surface compared to the wild type channel suggesting that reduced sodium channel density may contribute to the pathophysiology of this inherited epilepsy.

METHODS

Mutagenesis of human Nav1.2 cDNA

Full-length human Nav1.2 cDNA was obtained as a generous gift from M. Montal (Ahmed et al., 1992). The open reading frame was subcloned into the mammalian expression plasmid pCMV-Script, the 5' UTR was removed to improve expression, and two cloning errors were corrected (G334D and R1744G). Polymerase chain reaction (PCR) site-directed mutagenesis was used to engineer individual mutations into pCMV-Nav1.2 using a previously described method (primer sequences provided in Supplemental Table S1) (Lossin et al., 2002; Lossin et al., 2003; Ohmori et al., 2006; Rhodes et al., 2004). Three BFNIS-associated mutations

were constructed using codon usage typical of human tissues. Multiple attempts to construct other BFNIS-associated mutations were unsuccessful. Due to the tendency of neuronal voltage-gated sodium channel cDNAs to spontaneously mutate within bacterial culture, we propagated clones in STBL2 host cells (Invitrogen Corporation, Carlsbad, CA) at 30°C, and the entire open reading frame of each construct was completely sequenced to exclude polymerase errors and inadvertent mutations. A minimum of two recombinant clones of each mutation were evaluated functionally.

Heterologous expression of Nav1.2

Recombinant Nav1.2 was heterologously coexpressed in human tsA201 cells (HEK293 derivative stably transfected with SV40 large T antigen) with the human β_1 and β_2 voltage-gated sodium channel accessory subunits similar to our previous method of Nav1.1 expression (Lossin et al., 2002; Lossin et al., 2003; Ohmori et al., 2006; Rhodes et al., 2004). Cells were grown in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA), L-glutamine (2 mM) and penicillin-streptomycin (50 units/ml and 50 μ g/ml, respectively) in a humidified, 5% CO₂ atmosphere at 37°C. Only low passage number (< 15) cells were used. Expression of Nav1.2, β_1 and β_2 was achieved by transient transfection using Qiagen Superfect reagent (5 μ g of DNA was transfected at a plasmid mass ratio of 10:1:1 for α_2 : β_1 : β_2). The human β_1 and β_2 cDNAs were expressed from plasmids that contained separate coding sequences of the fluorescent proteins EGFP (h β_1 -IRES2-EGFP) or DsRed (h β_2 -IRES2-DsRed2) preceded by an internal ribosome entry site (IRES). Cells were plated on glass coverslips and only cells successfully transfected with β_1 and β_2 were used for electrophysiological studies. Unless otherwise noted, all reagents were purchased from Sigma Aldrich (Sigma, St Louis, MO, U.S.A.).

Electrophysiology and data analysis

Whole-cell voltage-clamp recordings were used to characterize the functional properties of WT and mutant sodium channels, as described previously (Lossin et al., 2002; Lossin et al., 2003; Ohmori et al., 2006; Rhodes et al., 2004). Sodium channel currents were recorded at room temperature, 48–72 h after transfection. Patch pipettes were fabricated from borosilicate glass (Warner Instrument Co., Hamden, CT, U.S.A) by a multistage P-97 Flaming-Brown micropipette puller (Sutter Instruments Co., San Rafael, CA, U.S.A.) and fire-polished by using a microforge (MF 830, Narashige, Japan). Pipette resistance was between 1.0 and 2.0 M Ω . The pipette solution consisted of (in mM) 110 CsF, 10 NaF, 20 CsCl, 2 EGTA, 10 HEPES, with a pH of 7.35 and osmolality of 310 mOsmol/kg. The bath solution contained in (mM): 145 NaCl, 4 KCl, 1.8 CaCl₂, 1 MgCl₂, 10 HEPES, with a pH of 7.35 and osmolality of 310 mOsmol/kg. The osmolality was adjusted with sucrose. The bath solution was continuously exchanged by a gravity-driven perfusion system. The reference electrode consisted of a 2% agar bridge with composition similar to the bath solution. Cells were allowed to stabilize for 15 min after establishment of the whole-cell configuration before current was measured. Cells exhibiting peak current amplitudes < 0.6 nA were excluded from analysis of biophysical properties to avoid contamination of recordings by a low amplitude (< 0.1 nA) endogenous sodium current that is sometimes present in tsA201 cells. Cells exhibiting peak current amplitudes > 6 nA were also generally excluded from analysis to ensure accurate voltage control. Whole-cell capacitance and access resistance were determined by integrating capacitive transients in response to voltage steps from –120 to –110 mV filtered at 10 kHz. Series resistance was compensated 90–95% to assure that the command potential was reached in less than 100 μ s with a voltage error < 2 mV. Leak currents were subtracted by using an online P/4 procedure. All data were low-pass Bessel filtered at 5 kHz and digitized at 50 kHz.

Specific voltage-clamp protocols assessing channel activation, voltage dependence of fast inactivation, and recovery from a 100 ms inactivating prepulse (recovery from fast inactivation) were used as described previously (Lossin et al., 2002; Lossin et al., 2003; Ohmori et al., 2006; Rhodes et al., 2004) and described by figure insets. All voltage-clamp protocols utilized a holding potential of -120 mV and a 60 s interpulse at the holding potential between sequential protocols. Voltage steps (20 ms) to between -80 to $+60$ mV in 10 mV increments were used to create a family of voltage-gated inward sodium current traces. The peak current was normalized for cell capacitance and plotted against voltage to generate peak current density-voltage relationships. Conductance (G_{Na}) was calculated as $G_{Na} = I/(V - E_{rev})$ where I is the measured peak current, V is the test voltage, and E_{rev} is the calculated sodium reversal potential. To provide a quantitative evaluation of the voltage dependence of activation, normalized G - V curves were fit with a Boltzmann function, $G/G_{max} = (1 + \exp[(V - V_{1/2})/k])^{-1}$, where $V_{1/2}$ is the curve midpoint indicating the voltage at which half of the channels are activated and k is a slope factor corresponding with voltage sensitivity of the channel. The time to peak current and 10–90% rise time were quantified for the -30 to $+30$ mV range using the same voltage-clamp protocol described above. Together these parameters define the magnitude of depolarization needed for channel opening. Voltage dependence of fast inactivation was assessed by 100 ms prepulses to between -160 to -10 mV in 10 mV increments followed by a 20 ms test pulse to -10 mV. The normalized current is plotted against the voltage and the data were fit with Boltzmann functions to determine the voltage for half-maximal inactivation ($V_{1/2}$) and a slope factor (k). Voltage dependence of fast inactivation provides information about the level of depolarization necessary for the channel to enter fast inactivation under steady-state conditions including the physiological range of potentials. Recovery from fast inactivation was determined using a two-pulse protocol. A 100 ms prepulse to -10 mV was followed by a variable amount of time for channel recovery and a 20 ms test pulse to -10 mV. The peak current from the test pulse was normalized to the peak current from the prepulse and plotted against the recovery period. Data were fit with the two exponential function, $I/I_{max} = A_f \times [1 - \exp(-t/\tau_f)] + A_s \times [1 - \exp(-t/\tau_s)]$, where τ_f and τ_s denote time constants (fast and slow components, respectively), and A_f and A_s represent the fast and slow fractional amplitudes. Time dependent recovery from inactivation provides information about how rapidly the channels can be available for a subsequent depolarization stimulus.

Inactivation of the whole-cell sodium current was evaluated by fitting the decay phase of the current with the two exponential function, $I/I_{max} = A_f \times \exp(-t/\tau_f) + A_s \times \exp(-t/\tau_s)$, where τ_f and τ_s denote time constants (fast and slow components, respectively), A_f and A_s represent the fast and slow fractional amplitudes. For use-dependence studies, cells were stimulated with depolarizing pulse trains (100 pulses, 5 ms, 0 mV) at the indicated frequencies. A recovery interval (15 s, -120 mV) followed each pulse train. Currents were normalized to the peak current recorded in response to the first pulse in each frequency train. Persistent current was evaluated during the final 10 ms of a 200 ms depolarization to -10 mV and expressed as a percentage of peak current following digital subtraction of currents recorded in the presence and absence of $10 \mu\text{M}$ tetrodotoxin (TTX). To prevent potential experimenter bias, persistent current experiments and analysis were performed blinded to genotype.

Results are presented as mean $\pm$ SEM. Statistical comparisons were made in reference to WT by using the unpaired Student's t test. Chi-square analysis was used to determine significance level for expression efficiency of mutant alleles compared to WT. One-way ANOVA analysis with a Newman-Keuls Multiple Comparison post-test was performed to determine significance for peak whole-cell inward current. Data analysis was performed using Clampfit 9.2 (Axon Instruments, Union City, CA, U.S.A), Excel 2002 (Microsoft,

Seattle, WA, U.S.A.), GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA), and OriginPro 7.0 (OriginLab, Northampton, MA, U.S.A) software.

Cell Surface Biotinylation

Cell surface biotinylation was performed as described previously (Carvelli et al., 2002; Daws et al., 2002; Kahlig et al., 2004; Kahlig et al., 2006), with minor modifications for Nav_v1.2 detection. Wild-type Nav_v1.2, R1319Q, L1330F, and L1563V were epitope tagged with a triple FLAG tag on the C-terminus to increase detection sensitivity. Cells transfected with WT or mutant Nav_v1.2, β_1 , and β_2 as described above were washed twice with 4°C PBS, then cell surface proteins were labeled with the cell membrane impermeant biotinylating reagent, Sulfo-NHS-Biotin (Pierce Biotechnology, Rockford, IL), for 1 hr. The reaction was quenched with 100 mM glycine. Cells were then lysed with RIPA buffer (150 mM NaCl, 50 mM Tris-base, 1% IGEPAL CA-630, 0.5% Na Deoxycholate, 0.1 % SDS, pH 7.5) supplemented with Complete Mini Protease Inhibitor Cocktail Tabs (Roche Applied Science, Indianapolis, IN). Scraped lysates were centrifuged at 16,000g for 30 min at 4°C. Biotinylated proteins in the supernatant were recovered by incubation (2 h at 4°C) with High Capacity Streptavidin Agarose beads (Pierce Biotechnology, Rockford, IL) followed by centrifugation. The beads were extensively washed with RIPA buffer and biotinylated proteins eluted with 2× Laemmli Sample Buffer containing fresh 5% β -mercaptoethanol. Proteins were separated using SDS-polyacrylamide (7.5%) gel electrophoresis and transferred to polyvinylidene difluoride membranes. Membranes were blocked at room temperature for two hours in 5% milk. Nav_v1.2 was detected with primary antibodies directed against the FLAG epitope (mouse, anti-FLAG M2, 1:15000, Sigma). The quantity of loaded protein in each lane was determined using a primary antibody directed against the endogenous protein transferrin (mouse, anti-human transferrin receptor, 1:10000 for total protein and 1:2500 for biotinylated protein, Zymed, Carlsbad, CA). Immunoreactive bands were visualized using horseradish peroxidase-conjugated secondary antibody (goat anti-mouse, 1:30000 for total protein and 1:10000 for biotinylated proteins, Santa Cruz Biotechnology, Santa Cruz, CA), directed against the primary antibody, ECL Plus (GE Healthcare, Buckinghamshire, UK) incubation and Hypersensitive ECL film detection. Protein band densitometry was performed using ImageJ software (NIH). To control for protein loading, each Nav_v1.2 band was normalized to the amount of endogenously expressed transferrin detected for each experimental condition. One-way ANOVA analysis with a Newman-Keuls Multiple Comparison post-test was performed to determine significance for cell surface biotinylation experiments.

RESULTS

We functionally characterized three BFNIS mutations (R1319Q, L1330F, and L1563V) using a recombinant human Nav_v1.2 co-expressed with the human β_1 and β_2 subunits in cultured cells (tsA201) of human origin. The three missense mutations have been reported previously; L1330F and L1563V by Heron et al. (Heron et al., 2002) and R1319Q by Berkovic et al. (Berkovic et al., 2004). Figure 1A illustrates the approximate position of each mutation within the predicted Nav_v1.2 two-dimensional topology.

BFNIS mutations cause reduced inward sodium current

All three mutations (R1319Q, L1330F, and L1563V) generated functional sodium channels when transiently expressed in tsA201 cells. Figure 1B illustrates representative whole-cell currents evoked by a series of depolarizing test potentials in cells transiently expressing wild-type Nav_v1.2 (WT), R1319Q, L1330F, or L1563V, while Fig. 2A presents the corresponding peak current density-voltage relationships. The percentage of tested cells exhibiting quantifiable sodium currents (defined as peak current > 0.6 nA) was significantly

lower for R1319Q and L1563V as compared to wild type (WT) channels (WT, 66%, $n = 174$; R1319Q, 41%, $n = 161$, $p < 0.001$; L1563V, 45%, $n = 115$, $p < 0.01$). In this analysis, L1330F expression was not significantly different than WT (61%, $n = 149$). However, when we examined the inward current from all tested cells, thus removing the selection bias for high $\text{Na}_v1.2$ expression, the data revealed that all three BFNIS mutants displayed significantly lower peak current amplitudes compared to WT. Wild-type channels exhibited a mean peak current of -1.96 nA ($n = 174$), compared to -0.81 nA for R1319Q ($n = 161$; $p < 0.001$), -1.52 nA for L1330F ($n = 149$; $p < 0.05$), and -1.12 nA for L1563V ($n = 115$; $p < 0.001$). Subsequent experiments assessing the kinetics and voltage dependences of activation and inactivation as well as the time course of recovery from inactivation utilized cells with peak inward sodium current > 0.6 nA. In the following subsections, specific biophysical properties of the three mutant channels are presented individually.

L1563V exhibits impaired inactivation

The BFNIS mutant L1563V affects a residue within the S2 transmembrane helix of the 4th domain (D4/S2, Fig. 1A). Activation properties (voltage dependence of activation, time to peak current, 10–90% rise time of activation) of L1563V were not significantly different than WT channels (Fig. 2B, 3B, Table 1 and Supplemental Table S2). However, L1563V appeared to exhibit slower whole-cell current decay (Fig. 1B). To quantify the time course of inactivation to enable statistical comparisons between WT and L1563V, the whole-cell current decay was fitted with a two-exponential equation and the time constant with the dominant amplitude (τ_f) was plotted against the test potential for L1563V and WT (Fig. 3A, fit parameters for all mutants provided in Table 2). For cells expressing L1563V we observed significantly larger time constants for inactivation during voltage steps between -10 and 10 mV suggesting impaired fast inactivation. In addition, the voltage dependence of fast inactivation following an inactivating prepulse was significantly shifted in the depolarizing direction for L1563V suggesting that this mutant resists entry into the fast inactivated state (Fig. 2C and Table 1). Furthermore, L1563V exhibited an accelerated recovery from fast inactivation indicated by a significantly smaller fast time constant (τ_f) (Fig. 2D and Table 1). These data illustrate that L1563V has impaired fast inactivation.

R1319Q has mixed activation and inactivation defects

The R1319Q mutation neutralizes a highly conserved arginine residue in the S4 voltage-sensing segment of domain 3 (Fig. 1A). This mutant exhibited a small but significant increase in the slower time constant for inactivation (τ_s) only at -30 mV (Table 2), but otherwise had inactivation kinetics that were not significantly different than WT channels. Voltage dependence of fast inactivation for R1319Q was also not significantly different from WT (Fig. 2C and Table 1), but R1319Q did exhibit a significant difference in recovery from fast inactivation (τ_f) compared to WT (Fig. 2D and Table 1). We observed a significantly greater time to peak current over the -10 to $+20$ mV range (Supplemental Table S2) for R1319Q channels as well as a significantly increased 10–90 % rise time of activation compared to WT (Fig. 3B). These findings demonstrated slower activation of this mutant. Further evidence for a defect in activation was reflected by a conductance-voltage relationship that was significantly shifted in the depolarizing direction for R1319Q as compared to WT (Fig. 2B, Table 1). The slope factor (k) also exhibited a significant difference suggesting less steep voltage dependence for R1319Q compared to WT. These differences are consistent with neutralization of an S4 positive charge important for voltage sensing (Stühmer et al., 1989). Our data suggest that the R1319Q mutation alters both inactivation and activation in a manner that predicts a net decrease in channel activity.

L1330F exhibits enhanced use-dependent behavior

The L1330F mutation affects the short intracellular S4–S5 linker connecting the voltage-sensing and central-pore regions of D3 (Heron et al., 2002; Long et al., 2005). Comparison of inactivation kinetics between WT and L1330F revealed significant differences in the time constant representing the minor (slow) component of inactivation but only at a few test voltages (Table 2). There were no statistical differences between L1330F and WT channels in the voltage dependence of activation, voltage dependence of fast inactivation, and recovery from fast inactivation (Fig. 2, Table 1). L1330F displayed minor differences in the 10–90% rise time and time to peak current compared to WT (Fig. 3B and Supplemental Table 2).

In the absence of major gating abnormalities for L1330F, we utilized a voltage protocol consisting of a train of depolarizing steps at varying frequencies to test for differences in use-dependent channel behavior. L1330F exhibited a significantly enhanced use-dependent loss of channel availability compared to WT over a large range of frequencies (22 to 133 Hz; Fig. 4A). By contrast, the other two mutations we studied exhibited no significant differences in channel availability during the same pulse protocol (Fig. 4A). The enhanced use-dependent loss of channel availability for L1330F was evident within the first several pulses as illustrated in Fig. 4B for pulsing frequencies of 22, 85, and 133 Hz. These data predict that L1330F will have a net reduction in channel function during rapid stimulation.

Absence of increased persistent current in BFNIS mutations

We previously observed that several $\text{Na}_v1.1$ mutations associated with various genetic epilepsy syndromes cause significantly increased persistent current (Lossin et al., 2002; Ohmori et al., 2006; Rhodes et al., 2005; Rhodes et al., 2004). However, none of the BFNIS mutants we studied exhibited this biophysical phenotype as compared to WT channels (magnitude of persistent current as percentage of peak current amplitude: WT: $1.64 \pm 0.35\%$, $n = 10$; R1319Q: $1.74 \pm 0.24\%$, $n = 9$; L1330F: $1.51 \pm 0.14\%$, $n = 7$; L1563V: $1.23 \pm 0.39\%$, $n = 7$). This finding suggests that increased persistent current is not a common biophysical hallmark of $\text{Na}_v1.2$ mutations associated with BFNIS.

BFNIS mutants exhibit reduced cell surface expression

All three BFNIS mutations exhibited significantly smaller mean whole-cell peak current levels as compared to WT $\text{Na}_v1.2$. We tested the hypothesis that these mutations affected the level of protein in the plasma membrane by using cell surface biotinylation. Figure 5A illustrates that each BFNIS mutant exhibits lower cell surface expression compared to WT. Quantification of cell surface $\text{Na}_v1.2$ protein expression from four independent experiments (Fig. 5B) demonstrated that, compared to WT, each mutant exhibits a substantial reduction in cell surface expression: 79% for R1319Q ($p < 0.001$), 47% for L1330F ($p < 0.01$), and 61% for L1563V ($p < 0.01$; $n = 4$). By contrast, there were no significant differences in total cellular protein levels for any of the mutants compared with WT (Supplemental Fig. 1). Reduced $\text{Na}_v1.2$ cell surface expression is a novel finding and this predicts a lower number of channels available for generating inward current, a potentially important factor in BFNIS pathogenesis.

DISCUSSION

Elucidation of the molecular basis for brain sodium channelopathies and establishment of genotype-phenotype correlations may shed light on epileptogenesis and help conceptualize new treatment strategies. In this paper, we examined the properties of three BFNIS mutations (R1319Q, L1330F, and L1563V) using the human $\text{Na}_v1.2$ α -subunit coexpressed with human β_1 and β_2 subunits. Although the clinical characteristics of patients with each of

these mutations were similar (Berkovic et al., 2004; Heron et al., 2002), our findings indicate that Nav1.2 mutations may exhibit a range of functional abnormalities as well as reduced cell surface expression signifying the complex molecular mechanisms underlying this inherited epilepsy.

In this study we examined the biophysical properties of WT and BFNIS mutant Nav1.2. The R1319Q mutation caused a depolarized and less steep voltage dependence of activation, slower activation, and delayed recovery from fast inactivation. These biophysical features predict a loss of channel function. L1330F decreased channel availability in response to repetitive stimulation over a large range of frequencies that may also indicate a loss of channel function. The L1563V mutation impaired fast inactivation, caused a depolarizing shift in the voltage dependence of fast inactivation and displayed accelerated recovery from fast inactivation. The functional characteristics of L1563V predict a net gain of channel function. In general, these various and subtle biophysical effects probably contribute to the pathogenesis of BFNIS but may not represent the entire story.

In addition to the functional defects conferred by BFNIS mutations, we observed that cells expressing mutant channels exhibited lower peak current levels compared to WT. Further, all three BFNIS mutants exhibited between 47 and 79% lower cell surface expression of channel protein compared to WT in tsA201 cells, and the relative magnitude of the reduction was concordant with mean whole-cell peak current levels. For example, R1319Q exhibited the largest decrease in mean whole-cell peak current as well as the lowest number of cells passing minimum current threshold, and this mutation exhibited the greatest decrement in cell surface expression. By contrast, L1330F had only a moderate reduction in both current amplitudes and cell surface expression. This finding suggests that reduced sodium channel density may be an important factor in the pathogenesis of this inherited epilepsy. Aberrant trafficking of the mutant proteins to the plasma membrane, possibly because of mis-folding or altered interactions with chaperone proteins including β -subunits, is the most plausible explanation for these findings (Hirose, 2006). Because tsA201 cells are not neuronal in origin, future studies in neurons may help confirm our findings and aid in elucidating the cellular mechanism of altered excitability in BFNIS.

SCN2A encodes Nav1.2, a voltage-gated sodium channel pore forming α -subunit expressed abundantly in the adult central nervous system. Knockout mice deficient in Nav1.2 die perinatally from neuronal apoptosis and hypoxia, suggesting that expression is crucial for early postnatal, but not embryonic development (Planells-Cases et al., 2000). Early in development, Nav1.2 is highly expressed in regions destined to become nodes of Ranvier, and is developmentally replaced by Nav1.6 in adult rat brains (Kaplan et al., 2001). Nav1.2 is also enriched at axon initial segments (AIS) in developing neurons (Boiko et al., 2003; Garrido et al., 2003b; Garrido et al., 2003a). In the adult brain, unmyelinated axons throughout the rostral central nervous system, including the cortex and hippocampus, have high Nav1.2 expression (Westenbroek et al., 1989; Westenbroek et al., 1992). Clusters of Nav1.2 at nodes and the AIS contribute to determining axonal firing frequency and action potential propagation (Boiko et al., 2003; Carras et al., 1992). Abnormal Nav1.2 function may disrupt the physiological role of this channel in controlling excitability and underlie pathogenesis of familial epilepsy syndromes. We speculate that the observed temporal transition of Nav1.2 to Nav1.6 (Salzer, 2002) may also be affected by abnormal Nav1.2 expression or activity.

The three BFNIS mutations presented in our study have been previously analyzed using cultured rat cortical neurons transfected with rat Nav1.2 (Scalmani et al., 2006). Differences in functional properties of the mutant channels between our study and that performed in rat neurons may be explained by species differences of the cDNAs, or experimental limitations

(e.g., uncertain voltage control) of the on-cell macropatch recording configuration applied to cultured neurons in that study. In addition, without a selective blocker of Na_v1.2, it is technically difficult to assign kinetic properties to Na_v1.2 expressed within the background of other voltage-gated sodium channels (Cummins et al., 2001; Herzog et al., 2003; Rush et al., 2005) especially if the mutant channels are expressed at very low levels as we have demonstrated. In our study, we used human Na_v1.2 coexpressed with the human β_1 and β_2 accessory subunits in cultured non-neuronal human cells. We further recorded Na_v1.2 activity using the whole-cell configuration of the patch-clamp allowing that enables more precise voltage and ionic control. This well established expression and recording approach uniquely permits the isolation and primary characterization of mutant human Na_v1.2 channels associated with BFNIS.

Another previous study proposed that the L1563V mutation preferentially affects a putative neonatal isoform of Na_v1.2 (Xu et al., 2007). Although, a preferential functional impact of a mutation on a developmentally regulated splice variant provides an intriguing explanation for the abatement of seizures during late infancy in BFNIS, this should be considered speculative because the temporal regulation of this splicing event is not fully defined (Kasai et al., 2001; Raymond et al., 2004; Sarao et al., 1991; Tate et al., 2005). Further, this previous study characterized Na_v1.2 in the absence of β subunits that are known modulators of neuronal sodium channel gating properties (Catterall, 2000; Catterall et al., 2005; Isom et al., 1994), and this limits an accurate correlation with native channels. The absence of β subunits may help to explain why the previous study did not observe significant effects of the L1563V mutation on the same Na_v1.2 splice variant that we used (Xu et al., 2007).

In summary, we have demonstrated that three *SCN2A* mutations associated with BFNIS cause subtle and divergent biophysical defects in Na_v1.2, but also impair cell surface expression. A greater understanding of the exact composition of sodium channel complexes *in vivo* and their targeting to different subcellular localizations will be required to fully understand how these defects cause BFNIS. Future studies will also need to account for the developmentally regulated role of Na_v1.2 in neuronal excitability and how this relates to the early seizure remittance seen in BFNIS. Animal models with genetically engineered Na_v1.2 mutations may help elucidate the physiological contribution of this isoform and further explain the pathogenesis of BFNIS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Ahmed CM, Ware DH, Lee SC, Patten CD, Ferrer-Montiel AV, Schinder AF, McPherson JD, Wagner-McPherson CB, Wasmuth JJ, Evans GA, Montal M. Primary structure, chromosomal localization, and functional expression of a voltage-gated sodium channel from human brain. *Proc Natl Acad Sci U S A*. 1992; 89:8220–8224. [PubMed: 1325650]
- Berkovic SF, Heron SE, Giordano L, Marini C, Guerrini R, Kaplan RE, Gambardella A, Steinlein OK, Grinton BE, Dean JT, Bordo L, Hodgson BL, Yamamoto T, Mulley JC, Zara F, Scheffer IE. Benign

- familial neonatal-infantile seizures: characterization of a new sodium channelopathy. *Ann Neurol*. 2004; 55:550–557. [PubMed: 15048894]
- Boiko T, Van WA, Caldwell JH, Levinson SR, Trimmer JS, Matthews G. Functional specialization of the axon initial segment by isoform-specific sodium channel targeting. *J Neurosci*. 2003; 23:2306–2313. [PubMed: 12657689]
- Carras PL, Coleman PA, Miller RF. Site of action potential initiation in amphibian retinal ganglion cells. *J Neurophysiol*. 1992; 67:292–304. [PubMed: 1569462]
- Carvelli L, Moron JA, Kahlig KM, Ferrer JV, Sen N, Lechleiter JD, Leeb-Lundberg LM, Merrill G, Lafer EM, Ballou LM, Shippenberg TS, Javitch JA, Lin RZ, Galli A. PI 3-kinase regulation of dopamine uptake. *J Neurochem*. 2002; 81:859–869. [PubMed: 12065645]
- Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron*. 2000; 26:13–25. [PubMed: 10798388]
- Catterall WA, Goldin AL, Waxman SG. International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. *Pharmacol Rev*. 2005; 57:397–409. [PubMed: 16382098]
- Charlier C, Singh NA, Ryan SG, Lewis TB, Reus BE, Leach RJ, Leppert M. A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. *Nat Genet*. 1998; 18:53–55. [PubMed: 9425900]
- Cummins TR, Aglieco F, Renganathan M, Herzog RI, Dib-Hajj SD, Waxman SG. Nav1.3 sodium channels: rapid repriming and slow closed-state inactivation display quantitative differences after expression in a mammalian cell line and in spinal sensory neurons. *J Neurosci*. 2001; 21:5952–5961. [PubMed: 11487618]
- Daws LC, Callaghan PD, Moron JA, Kahlig KM, Shippenberg TS, Javitch JA, Galli A. Cocaine increases dopamine uptake and cell surface expression of dopamine transporters. *Biochem Biophys Res Commun*. 2002; 290:1545–1550. [PubMed: 11820798]
- Garrido JJ, Fernandes F, Moussif A, Fache MP, Giraud P, Dargent B. Dynamic compartmentalization of the voltage-gated sodium channels in axons. *Biol Cell*. 2003a; 95:437–445. [PubMed: 14597261]
- Garrido JJ, Giraud P, Carlier E, Fernandes F, Moussif A, Fache MP, Debanne D, Dargent B. A targeting motif involved in sodium channel clustering at the axonal initial segment. *Science*. 2003b; 300:2091–2094. [PubMed: 12829783]
- Harkin LA, McMahon JM, Iona X, Dibbens L, Pelekanos JT, Zuberi SM, Sadleir LG, Andermann E, Gill D, Farrell K, Connolly M, Stanley T, Harbord M, Andermann F, Wang J, Batish SD, Jones JG, Seltzer WK, Gardner A, Sutherland G, Berkovic SF, Mulley JC, Scheffer IE. The spectrum of SCN1A-related infantile epileptic encephalopathies. *Brain*. 2007; 130:843–852. [PubMed: 17347258]
- Herlenius E, Heron SE, Grinton BE, Keay D, Scheffer IE, Mulley JC, Berkovic SF. SCN2A mutations and benign familial neonatal-infantile seizures: the phenotypic spectrum. *Epilepsia*. 2007; 48:1138–1142. [PubMed: 17386050]
- Heron SE, Crossland KM, Andermann E, Phillips HA, Hall AJ, Bleasel A, Shevell M, Mercho S, Seni MH, Guiot MC, Mulley JC, Berkovic SF, Scheffer IE. Sodium-channel defects in benign familial neonatal-infantile seizures. *Lancet*. 2002; 360:851–852. [PubMed: 12243921]
- Herzog RI, Cummins TR, Ghassemi F, Dib-Hajj SD, Waxman SG. Distinct repriming and closed-state inactivation kinetics of Nav1.6 and Nav1.7 sodium channels in mouse spinal sensory neurons. *J Physiol*. 2003; 551:741–750. [PubMed: 12843211]
- Hirose S. A new paradigm of channelopathy in epilepsy syndromes: intracellular trafficking abnormality of channel molecules. *Epilepsy Res*. 2006; 70(Suppl 1):S206–S217. [PubMed: 16860540]
- Isom LL, De Jongh KS, Catterall WA. Auxiliary subunits of voltage-gated ion channels. *Neuron*. 1994; 12:1183–1194. [PubMed: 7516685]
- Kahlig KM, Javitch JA, Galli A. Amphetamine regulation of dopamine transport. Combined measurements of transporter currents and transporter imaging support the endocytosis of an active carrier. *J Biol Chem*. 2004; 279:8966–8975. [PubMed: 14699142]

- Kahlig KM, Lute BJ, Wei Y, Loland CJ, Gether U, Javitch JA, Galli A. Regulation of dopamine transporter trafficking by intracellular amphetamine. *Mol Pharmacol*. 2006; 70:542–548. [PubMed: 16684900]
- Kamiya K, Kaneda M, Sugawara T, Mazaki E, Okamura N, Montal M, Makita N, Tanaka M, Fukushima K, Fujiwara T, Inoue Y, Yamakawa K. A nonsense mutation of the sodium channel gene *SCN2A* in a patient with intractable epilepsy and mental decline. *J Neurosci*. 2004; 24:2690–2698. [PubMed: 15028761]
- Kaplan MR, Cho MH, Ullian EM, Isom LL, Levinson SR, Barres BA. Differential control of clustering of the sodium channels $\text{Nav}1.2$ and $\text{Nav}1.6$ at developing CNS nodes of Ranvier. *Neuron*. 2001; 30:105–119. [PubMed: 11343648]
- Kaplan RE, Lacey DJ. Benign familial neonatal-infantile seizures. *Am J Med Genet*. 1983; 16:595–599. [PubMed: 6660252]
- Kasai N, Fukushima K, Ueki Y, Prasad S, Nosakowski J, Sugata K, Sugata A, Nishizaki K, Meyer NC, Smith RJ. Genomic structures of *SCN2A* and *SCN3A* - candidate genes for deafness at the *DFNA16* locus. *Gene*. 2001; 264:113–122. [PubMed: 11245985]
- Long SB, Campbell EB, Mackinnon R. Crystal structure of a mammalian voltage-dependent Shaker family K^+ channel. *Science*. 2005; 309:897–903. [PubMed: 16002581]
- Lossin C, Rhodes TH, Desai RR, Vanoye CG, Wang D, Carniciu S, Devinsky O, George AL Jr. Epilepsy-associated dysfunction in the voltage-gated neuronal sodium channel *SCN1A*. *J Neurosci*. 2003; 23:11289–11295. [PubMed: 14672992]
- Lossin C, Wang DW, Rhodes TH, Vanoye CG, George AL Jr. Molecular basis of an inherited epilepsy. *Neuron*. 2002; 34:877–884. [PubMed: 12086636]
- Meisler MH, Kearney JA. Sodium channel mutations in epilepsy and other neurological disorders. *J Clin Invest*. 2005; 115:2010–2017. [PubMed: 16075041]
- Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I, Takeuchi T, Itohara S, Yanagawa Y, Obata K, Furuichi T, Hensch TK, Yamakawa K. $\text{Nav}1.1$ localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an *Scn1a* gene mutation. *J Neurosci*. 2007; 27:5903–5914. [PubMed: 17537961]
- Ohmori I, Kahlig KM, Rhodes TH, Wang DW, George AL Jr. Nonfunctional *SCN1A* is common in severe myoclonic epilepsy of infancy. *Epilepsia*. 2006; 47:1636–1642. [PubMed: 17054685]
- Planells-Cases R, Caprini M, Zhang J, Rockenstein EM, Rivera RR, Murre C, Masliah E, Montal M. Neuronal death and perinatal lethality in voltage-gated sodium channel α_{II} -deficient mice. *Biophys J*. 2000; 78:2878–2891. [PubMed: 10827969]
- Raymond CK, Castle J, Garrett-Engle P, Armour CD, Kan Z, Tsinoremas N, Johnson JM. Expression of alternatively spliced sodium channel α -subunit genes. Unique splicing patterns are observed in dorsal root ganglia. *J Biol Chem*. 2004; 279:46234–46241. [PubMed: 15302875]
- Rhodes TH, Lossin C, Vanoye CG, Wang DW, George AL Jr. Noninactivating voltage-gated sodium channels in severe myoclonic epilepsy of infancy. *Proc Natl Acad Sci U S A*. 2004; 101:11147–11152. [PubMed: 15263074]
- Rhodes TH, Vanoye CG, Ohmori I, Ogiwara I, Yamakawa K, George AL Jr. Sodium channel dysfunction in intractable childhood epilepsy with generalized tonic-clonic seizures. *J Physiol*. 2005; 569:433–445. [PubMed: 16210358]
- Rush AM, Dib-Hajj SD, Waxman SG. Electrophysiological properties of two axonal sodium channels, $\text{Nav}1.2$ and $\text{Nav}1.6$, expressed in mouse spinal sensory neurones. *J Physiol*. 2005; 564:803–815. [PubMed: 15760941]
- Salzer JL. Nodes of Ranvier come of age. *Trends Neurosci*. 2002; 25:2–5. [PubMed: 11801321]
- Sarao R, Gupta SK, Auld VJ, Dunn RJ. Developmentally regulated alternative RNA splicing of rat brain sodium channel mRNAs. *Nucleic Acids Res*. 1991; 19:5673–5679. [PubMed: 1658739]
- Scalmani P, Rusconi R, Armatura E, Zara F, Avanzini G, Franceschetti S, Mantegazza M. Effects in neocortical neurons of mutations of the $\text{Nav}1.2$ Na^+ channel causing benign familial neonatal-infantile seizures. *J Neurosci*. 2006; 26:10100–10109. [PubMed: 17021166]
- Singh NA, Charlier C, Stauffer D, DuPont BR, Leach RJ, Melis R, Ronen GM, Bjerre I, Quattlebaum T, Murphy JV, McHarg ML, Gagnon D, Rosales TO, Peiffer A, Anderson VE, Leppert M. A

- novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. *Nat Genet.* 1998; 18:25–29. [PubMed: 9425895]
- Striano P, Bordo L, Lispi ML, Specchio N, Minetti C, Vigeveno F, Zara F. A novel SCN2A mutation in family with benign familial infantile seizures. *Epilepsia.* 2006; 47:218–220. [PubMed: 16417554]
- Stühmer W, Conti F, Suzuki H, Wang XD, Noda M, Yahagi N, Kubo H, Numa S. Structural parts involved in activation and inactivation of the sodium channel. *Nature.* 1989; 339:597–603. [PubMed: 2543931]
- Sugawara T, Tsurubuchi Y, Agarwala KL, Ito M, Fukuma G, Mazaki-Miyazaki E, Nagafuji H, Noda M, Imoto K, Wada K, Mitsudome A, Kaneko S, Montal M, Nagata K, Hirose S, Yamakawa K. A missense mutation of the Na⁺ channel α_{II} subunit gene Nav1.2 in a patient with febrile and afebrile seizures causes channel dysfunction. *Proc Natl Acad Sci U S A.* 2001; 98:6384–6389. [PubMed: 11371648]
- Tate SK, Depondt C, Sisodiya SM, Cavalleri GL, Schorge S, Soranzo N, Thom M, Sen A, Shorvon SD, Sander JW, Wood NW, Goldstein DB. Genetic predictors of the maximum doses patients receive during clinical use of the anti-epileptic drugs carbamazepine and phenytoin. *Proceedings of the National Academy of Sciences USA.* 2005; 102:5507–5512.
- Westenbroek RE, Merrick DK, Catterall WA. Differential subcellular localization of the RI and RII Na⁺ channel subtypes in central neurons. *Neuron.* 1989; 3:695–704. [PubMed: 2561976]
- Westenbroek RE, Noebels JL, Catterall WA. Elevated expression of type II Na⁺ channels in hypomyelinated axons of *shiverer* mouse brain. *J Neurosci.* 1992; 12:2259–2267. [PubMed: 1318958]
- Xu R, Thomas EA, Jenkins M, Gazina EV, Chiu C, Heron SE, Mulley JC, Scheffer IE, Berkovic SF, Petrou S. A childhood epilepsy mutation reveals a role for developmentally regulated splicing of a sodium channel. *Mol Cell Neurosci.* 2007; 35:292–301. [PubMed: 17467289]
- Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, McKnight GS, Scheuer T, Catterall WA. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nat Neurosci.* 2006; 9:1142–1149. [PubMed: 16921370]

Figure 1. Representative WT and mutant Nav1.2 whole-cell sodium currents
(A) Predicted transmembrane topology of Nav1.2 showing the location of BFNIS mutations as circles (open circles represent mutations characterized in this study). **(B)** Sodium currents recorded from tsA201 cells co-expressing the indicated Nav1.2 allele with the β_1 and β_2 accessory subunits. Currents were activated by voltage steps to between -80 and $+60$ mV from a holding potential of -120 mV (see pulse protocol inset).

Figure 2. Biophysical properties of WT and mutant Nav1.2

Biophysical properties of human WT, R1319Q, L1330F, and L1563V expressed in human tsA201 cells. **(A)** Peak current density elicited by test pulses to various potentials and normalized to cell capacitance. **(B)** Voltage dependence of channel activation measured during voltage steps to between -80 and $+20$ mV. R1319Q displayed a significant depolarizing shift in activation compared to WT. **(C)** Voltage dependence of fast inactivation assessed in response to inactivating prepulses to between -160 and -10 mV. L1563V displayed a significant depolarizing shift in the voltage dependence of fast inactivation compared to WT. **(D)** Time dependent recovery from fast inactivation assessed following an inactivating prepulse (100 ms at -10 mV). Significant defects in the recovery were observed for both R1319Q (delayed recovery) and L1563V (accelerated recovery). Pulse protocols are shown as panel insets and fit parameters are provided in Table 1.

Figure 3. Inactivation and activation kinetics for WT and mutant Nav1.2

(A) Fast inactivation time constants for WT and BFNIS-associated mutants plotted against test potential. Fast time constants were significantly larger for L1563V (between -10 to $+10$ mV, $p < 0.05$) compared to WT channels. Fit parameters for all mutants are provided in Table 2. (B) Activation kinetics assessed by 10–90% rise time plotted against test potential for WT and BFNIS-associated mutants. The rise time was significantly longer for R1319Q over the -10 to $+20$ mV range ($p < 0.05$) and significantly shorter for L1330F at -10 mV ($p < 0.05$) compared to WT.

Figure 4. Use-dependent behavior of WT and mutant Nav1.2

The response of WT and L1330F to repetitive depolarization (use-dependence) was measured by stimulating cells with voltage step pulse trains (100 pulses, 5 ms, 0 mV) from a holding potential of -120 mV at the indicated frequencies (see inset in panel A). **(A)** Residual peak current amplitude of the 25th pulse for WT, R1319Q, L1330F and L1563V plotted against pulse frequency. Fewer L1330F channels were available for activation in response to the 25th pulse compared to WT for the stimulation frequencies of between 22 and 133 Hz (*, $p < 0.05$; **, $p < 0.01$). **(B)** Normalized peak current measured in response to a voltage step train at frequencies of 22, 85, and 133 Hz. **L1330F displays significantly decreased residual current for pulses 2 and 5 to 100 for 22 Hz, pulses 8 to 100 for 85 Hz, and pulses 2 to 100 for 133 Hz ($p \leq 0.05$).**

Figure 5. Reduced cell surface protein expression of BFNIS mutants

Cell surface expression of WT and the three BFNIS mutations was measured using cell surface biotinylation. Endogenous transferrin levels were measured as a gel loading control. (A) Representative experiment illustrating total (top) and cell surface (bottom) Na_v1.2 protein detected with anti-FLAG antibody. Na_v1.2 immunoreactive bands were normalized to the amount of an endogenous protein (transferrin) detected in each experimental lane (below Na_v1.2 detection). The first lane was isolated from cells transfected with untagged WT. Lanes 2 – 5 are the FLAG tagged Na_v1.2 constructs (WT_{FLAG}, R1319Q_{FLAG}, L1330F_{FLAG}, L1563V_{FLAG}, respectively). Lane 6 was isolated from untransfected tsA201 cells (mock). All three BFNIS mutations exhibited decreased cell surface Na_v1.2 protein expression. (B) Quantification of four independent experiments demonstrated that each BFNIS mutant exhibits a significantly lower level of cell surface channel expression: R1319Q is 21% of WT (***, $p < 0.001$), L1330F is 53% of WT (**, $p < 0.01$) and L1563V is 39% of WT (**, $p < 0.01$) ($n = 4$ for WT, R1319Q, L1330F, and L1563V). The biotinylated and total Na_v1.2 bands were normalized to the corresponding transferrin band and the ratio of the normalized biotinylated to total are shown for WT, R1319Q, L1330F and L1563V.

Table 1

Biophysical parameters of SCN2A BENIS

	Voltage dependence of activation			Voltage dependence of fast inactivation			Recovery from 100 ms inactivating prepulse		
	$V_{1/2}$ (mV)	k (mV)	n	$V_{1/2}$ (mV)	k (mV)	n	τ_f (ms) [§]	τ_s (ms) [§]	n
WT	-25.3 ± 1.4	7.5 ± 0.4	14	-67.4 ± 1.7	9.1 ± 0.8	14	1.4 ± 0.1 (75 ± 2%)	53.6 ± 6.9 (24 ± 2%)	16
R1319Q	-21.4 ± 0.9*	8.6 ± 0.2*	15	-67.1 ± 1.0	10.4 ± 0.9	16	1.7 ± 0.1* (74 ± 2%)	69.8 ± 13.0 (25 ± 2%)	15
L1330F	-25.5 ± 0.7	7.5 ± 0.2	15	-69.7 ± 1.0	8.2 ± 0.4	18	1.5 ± 0.1 (79 ± 2%)	65.5 ± 7.4 (21 ± 2%)	16
L1563V	-27.4 ± 1.8	6.5 ± 0.3	16	-62.9 ± 1.1*	8.1 ± 0.3	16	1.0 ± 0.1*** (80 ± 1%)*	63.6 ± 8.3 (19 ± 1%)*	16

Values significantly different from WT are indicated as follows

* $p < 0.05$,

*** $p < 0.001$.

[§] values in parentheses are amplitude.

Table 2

Whole-cell current inactivation time constants (τ)

	Fast Component [§]			Slow Component [§]				
	WT	R1319Q	L1330F	L1563V	WT	R1319Q	L1330F	L1563V
-30 mV	1.06 ± 0.16 (81 ± 9%)	1.03 ± 0.12 (81 ± 6%)	1.00 ± 0.08 (90 ± 3%)	1.22 ± 0.17 (81 ± 6%)	4.3 ± 0.2 (19 ± 9%)	5.4 ± 0.2* (20 ± 1%)	6.4 ± 0.9* (11 ± 3%)	4.8 ± 0.9 (19 ± 5%)
-20 mV	0.65 ± 0.06 (91 ± 2%)	0.75 ± 0.06 (90 ± 3%)	0.62 ± 0.03 (95 ± 1%)	0.84 ± 0.08 (90 ± 1%)	3.6 ± 0.3 (9 ± 3%)	3.7 ± 0.6 (10 ± 3%)	5.1 ± 0.7 (5 ± 0.4%)	4.3 ± 0.5 (10 ± 1%)
-10 mV	0.47 ± 0.04 (93 ± 1%)	0.50 ± 0.04 (93 ± 1%)	0.43 ± 0.02 (96 ± 1%)	0.63 ± 0.04* (95 ± 1%)	3.3 ± 0.3 (7 ± 1%)	4.0 ± 0.5 (7 ± 1%)	5.0 ± 0.5* (4 ± 1%)	4.1 ± 0.4 (5 ± 1%)
0 mV	0.39 ± 0.03 (94 ± 1%)	0.41 ± 0.02 (94 ± 1%)	0.33 ± 0.02 (96 ± 1%)	0.47 ± 0.02* (94 ± 2%)	3.3 ± 0.5 (6 ± 1%)	4.0 ± 0.5 (6 ± 1%)	4.1 ± 0.6 (4 ± 1%)	3.6 ± 0.5 (6 ± 2%)
10 mV	0.31 ± 0.03 (94 ± 1%)	0.35 ± 0.02 (93 ± 1%)	0.27 ± 0.01 (95 ± 2%)	0.39 ± 0.01* (95 ± 1%)	3.0 ± 0.4 (6 ± 1%)	3.2 ± 0.7 (7 ± 1%)	3.9 ± 0.4* (5 ± 2%)	3.6 ± 0.6 (5 ± 1%)
20 mV	0.27 ± 0.02 (93 ± 2%)	0.32 ± 0.02 (92 ± 0.4%)	0.24 ± 0.01 (94 ± 3%)	0.32 ± 0.01 (95 ± 1%)	3.0 ± 0.4 (7 ± 2%)	2.7 ± 0.1 (7 ± 0.4%)	3.7 ± 0.4 (6 ± 3%)	3.9 ± 0.5 (5 ± 1%)
30 mV	0.26 ± 0.02 (93 ± 1%)	0.29 ± 0.02 (89 ± 2%)	0.23 ± 0.01 (94 ± 3%)	0.29 ± 0.01 (93 ± 1%)	2.4 ± 0.2 (7 ± 1%)	2.6 ± 0.1 (10 ± 2%)	3.9 ± 0.4 (6 ± 3%)	3.0 ± 0.5

Values significantly different from WT-SCN2A are indicated as follows *

$p < 0.05$.

[§] values in parentheses are amplitude.