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Epilepsy-Associated Dysfunction in the Voltage-Gated Neuronal Sodium Channel SCN1A

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Mutations in SCN1A, the gene encoding the brain voltage-gated sodium channel α1 subunit (NaV1.1), are associated with at least two forms of epilepsy, generalized epilepsy with febrile seizures plus (GEFS +) and severe myoclonic epilepsy of infancy (SMEI). We examined the functional properties of four GEFS + alleles and one SMEI allele using whole-cell patch-clamp analysis of heterologously expressed recombinant human SCN1A. One previously reported GEFS + mutation (I1656M) and an additional novel allele (R1657C), both affecting residues in a voltage-sensing S4 segment, exhibited a similar depolarizing shift in the voltage dependence of activation. Additionally, R1657C showed a 50% reduction in current density and accelerated recovery from slow inactivation. Unlike three other GEFS + alleles that we recently characterized, neither R1657C nor I1656M gave rise to a persistent, noninactivating current. In contrast, two other GEFS + mutations (A1685V and V1353L) and L986F, an SMEI-associated allele, exhibited complete loss of function. In conclusion, our data provide evidence for a wide spectrum of sodium channel dysfunction in familial epilepsy and demonstrate that both GEFS + and SMEI can be associated with nonfunctional SCN1A alleles.

Key words: epilepsy; sodium channel; GEFS +; SMEI; SCN1A; electrophysiology

Introduction

Voltage-gated sodium channels are responsible for the generation and propagation of action potentials in excitable tissues. Mutations in genes encoding sodium channel pore-forming α subunits and an accessory β subunit have been associated with disorders of membrane excitability, including inherited forms of epilepsy (Ptacek, 2002). Two distinct clinical syndromes with overlapping features have been linked recently to mutations in the neuronal sodium channel α1 subunit gene SCN1A, and a more complete understanding of the precise molecular and biophysical mechanisms underlying these disorders is needed.

In generalized epilepsy with febrile seizures plus (GEFS +), convulsions associated with fever continue to occur beyond the age when typical febrile seizures abate (Scheffer and Berkovic, 1997). Affected individuals with GEFS + may also experience afebrile ictal episodes of varying types, including generalized tonic-clonic, absence, myoclonic, atonic, and myoclonic–astatic seizures. Missense mutations in SCN1A predominate among the genetic defects associated with GEFS + (Escayg et al., 2000, 2001; Abou-Khalil et al., 2001; Sugawara et al., 2001a; Wallace et al., 2001b), but heritable alterations in two other sodium channel genes (SCN1B and SCN2A) (Wallace et al., 1998; Sugawara et al., 2001b) and a GABA receptor subunit (GABRG2) (Baulac et al., 2001; Wallace et al., 2001a) have been implicated as well. In most cases, GEFS + is considered to be a benign disorder.

In contrast, severe myoclonic epilepsy of infancy (SMEI), also known as Dravet syndrome (Online Mendelian Inheritance in Man 607208), is a rare convulsive disorder characterized by febrile seizures with onset during the first year of life, followed by intractable epilepsy, impaired psychomotor development, and ataxia (Dravet and Bureau, 1981; Scheffer et al., 2001). Seizures in this disorder are usually unresponsive to anticonvulsant drugs. Recently, several de novo heterozygous SCN1A mutations have been reported in SMEI probands including missense, nonsense, and insertion/deletion alleles (Claes et al., 2001, 2003; Ohmori et al., 2002; Sugawara et al., 2002; Fujiwara et al., 2003; Nabbout et al., 2003). The observed clinical similarities between SMEI and GEFS +, including the frequent occurrence of febrile seizures and shared molecular genetic etiology, have prompted the idea that these disorders represent two extremes in clinical presentation of the same condition (Singh et al., 2001).

To improve our understanding of the molecular basis of GEFS + and SMEI specifically, and to broaden our knowledge of epileptogenesis in general, we and others have begun characterizing the biophysical properties of mutant voltage-gated sodium channels associated with these disorders (Alekov et al., 2000, 2001; Spampanato et al., 2001, 2003; Lossin et al., 2002; Cossette et al., 2003). Recently, we reported that three SCN1A mutations associated with GEFS + caused similar defects in fast inactivation gating characterized by a persistent, noninactivating current during membrane depolarizations when recombinant human SCN1A was coexpressed heterologously with its accessory subunit.
units (Lossin et al., 2002). Our findings suggested that, in some cases of GEFS+, SCN1A mutations may promote a gain of function in sodium channels, leading to neuronal hyperexcitability. Whether all SCN1A mutations associated with GEFS+ exhibit the same pattern of channel dysfunction has not been determined.

We have now characterized four additional SCN1A missense mutations associated with GEFS+, including a novel allele and one missense mutation reported in an SMEI proband. Surprisingly, two of the four GEFS+ mutations, as well as the SMEI mutation, exhibited no function in heterologous cells. In contrast, the other two GEFS+ alleles were functional but had altered gating properties. Our findings suggest that a spectrum of functional sodium channel defects rather than a single biophysical mechanism contribute to the pathogenesis of inherited epilepsy.

Materials and Methods

Mutation screening. Family NY-005 is a small, three-generation pedigree with Ashkenazi Jewish heritage ascertained in the New York City area. The proband (male) developed generalized tonic–clonic seizures associated with high fever at 2 years of age. Over the subsequent 18 months, he experienced at least three additional generalized febrile convulsions generally lasting 2–5 min. In addition, he experienced episodic myoclonic jerks associated with momentary impairment of consciousness and conjugate upward eye deviation. The frequency of these episodes was variable, but fatigue, loud noise, or sudden bright light exposure were identified as possible triggering events. The proband’s mother, maternal grandmother, and a maternal aunt all experienced febrile convulsions during childhood, although the age of termination of febrile seizures is unknown. This family was diagnosed with probable GEFS+.

Genomic DNA (25 ng) was screened for SCN1A mutations using PCR–single-strand conformational analysis, as described previously. Mutation R1657C was independently verified using allele-specific oligonucleotide hybridization coupled with chemiluminescent detection (Pierce, Rockford, IL). The probe sequence was 5’-ATCCTGACGGCTGCT-biotin-3’ (mutant base italicized). Hybridizations and washes were performed at 47.5°C.

Mutagenesis and heterologous expression of human SCN1A. Full-length human SCN1A (Na1,1) cDNA was prepared and mutated as described previously (Lossin et al., 2002). To reconstitute the native brain sodium channel complex, SCN1A was coexpressed heterologously with human accessory subunits in HEK293 and tsA201 cells, as described previously (Lossin et al., 2002). All mutant cDNAs were fully sequenced before their use in transfection experiments. At least two different recombinant clones of each mutant cDNA were evaluated. Concentration of each plasmid DNA was determined spectrophotometrically (UV) as well as fluorometrically (Picogreen; Molecular Probes, Eugene, OR).

Electrophysiology and data analysis. Whole-cell voltage-clamp experiments addressing fast inactivation were described previously (Lossin et al., 2002). Whole-cell capacitance and access resistance were determined by integrating capacitive transients of voltage steps from −120 to −10 mV filtered at 10 kHz. Series resistance (2 ± 0.1 MΩ) was compensated 87–95% to assure that the command potential was reached within microseconds and with a voltage error <3 mV. All data were acquired at 10–50 kHz and low-pass Bessel filtered at 5 kHz. Persistent current was evaluated by 100 msec depolarizations to −10 mV in the presence and absence of 10 μM TTX, a potent sodium channel blocker (Sigma, St. Louis, MO). Representative traces for each condition were selected to have the same initial leak current, a composite TTX-sensitive trace was generated, and the mean current of the composite trace between 80 and 100 msec was calculated as percentage of the peak current. Data of recovery and steady-state inactivation experiments were digitally adjusted for leak current by subtracting the mean inward current apparent at 15–20 msec in a −10 mV step. Recording artifacts were further avoided by selecting traces with rapid capacitive transients and a holding current at −120 mV of <5% of the peak current. The range of peak current amplitude recorded at −10 mV in this study was 0.8–11.3 nA.

Slow inactivation was examined by paired-pulse protocols in which the response to a 50 msec test pulse to −10 mV was measured before (P1) and after (P2) variable conditioning protocols. Current measured during P2 was then normalized to the maximal current measured during P1. In detail, for onset of slow inactivation, the conditioning comprised voltage steps to −10 mV lasting from 100 msec to 100 sec, followed by a 50 msec recovery from fast inactivation at −120 mV. Conditioning for steady-state slow inactivation consisted of a 30 sec step to voltages between −140 and −10 mV and a 50 msec fast inactivation recovery at −120 mV. Conditioning for recovery from slow inactivation experiments used a 30 sec pulse to −10 mV, followed by recovery periods ranging from 100 msec to 100 sec. P1 pulses were repeated before each change of condition to minimize offset from run-down. In all cases, sufficient recovery time at −120 mV was allotted between different conditions to avoid accumulation of slow inactivation (determined in preliminary experiments; data not shown). Recordings with noticeable changes in P1 amplitude during the course of the experiment were discarded. Data from onset of slow inactivation experiments were plotted versus the length of the conditioning pulse and fitted to the two-exponential decay function:

\[ f(x) = y_0 + A_1 \cdot e^{-x/t_1} + A_2 \cdot e^{-x/t_2}, \]

where \( A_1 \) describes the fraction of the channels entering slow inactivation with time constant \( t_1 \), and \( y_0 \) describes fraction of the channels that remains active. Steady-state slow inactivation data were fitted to the Boltzmann function:

\[ f(x) = \frac{1 - y_0}{1 + e^{(x - V)/k}} + y_0, \]

where \( V_I/2 \) is the voltage at which half-maximal slow inactivation occurs, \( k \) is the slope factor of the fit, and \( y_0 \) is the fraction of the channels that remains active. Recovery from slow inactivation was fitted to the two-exponential function:

\[ f(x) = A_1 \cdot (1 - e^{-x/t_1}) + A_2 \cdot (1 - e^{-x/t_2}), \]

where \( A_1 \) refers the fraction of the channels recovering with time constant \( t_1 \). Data analysis was performed using Clampfit 8.2 (Axon Instruments, Union City, CA), Excel 2002 (Microsoft, Seattle, WA), and OriginPro 7.0 (OriginLab, Northampton, MA) software. All data were fit using a nonlinear least squares minimization method. Results are presented as means ± SE, and statistical comparisons were made between data from mutant sodium channels and wild-type (WT) SCN1A using the unpaired Student’s t test. Statistical significance was assumed for \( p < 0.05 \) and deemed not significant (NS) for \( p \geq 0.05 \). In some figures, the SE bars are smaller than the data symbols.

Single-channel current measurements were performed in the outside-out patch configuration at room temperature using the same solutions as the whole-cell experiments. Pipettes were pulled from thick-wall borosilicate glass (World Precision Instruments, Sarasota, FL) with a multistage P-97 Flaming–Brown micropipette puller (Sutter Instruments, San Rafael, CA). Fire-polished patch pipettes for single-channel studies were additionally coated with Sylgard 184 (Dow Corning, Midland, MI) and had resistance of −6 MΩ with standard pipette and bath solutions. Single-channel conductance was estimated by fitting the single-channel amplitude–voltage relationships in the range −30 to +10 mV by linear regression.

Rescue experiments. To test for rescue of trafficking-defective mutants, tsA201 cells were transfected with L986F plasmid DNA and both β subunits at 37°C, as described above. Immediately after the transfection, the medium was replaced with drug-supplemented media or the cells were transferred to a low temperature (26°C) incubator. The cells were washed three times with prewarmed DMEM and incubated in drug-free media for another 1.5 hr at 37°C before data acquisition. Low temperature rescue only was analogously attempted for V1353L and A1685V.

Results

A novel SCN1A mutation associated with epilepsy

We screened a family with Ashkenazi Jewish heritage segregating probable GEFS+ and discovered a novel C→T transition at nu-
sodium channel subunits expressed heterologously with the recombinant human accessory demonstrated to exhibit abnormal inactivation (Lossin et al., 2001b) and in close proximity to R1648H, an SCN1A mutation originally described by Escayg et al. (2000) that we recently observed in individuals of Ashkenazi descent. The nucleotide change predicts the replacement of arginine-1657 with cysteine in the S4 segment of domain IV (D4/S4), part of the sodium channel voltage-sensing apparatus. The replaced arginine is the innermost positively charged residue in D4/S4 and is highly conserved among vertebrate and invertebrate sodium channels. We designated this disturbance in activation and inactivation

Because both R1657C and I1656M affect conserved residues in a voltage–sensor domain (D4/S4 segment), we assessed the voltage dependence of activation and discovered that both mutants caused significant depolarizing shifts in this relationship. The voltage at which channels were half-maximally activated was −23.6 ± 1.2 mV (n = 17) for WT channels and −17.4 ± 1.5 mV (n = 8; p < 0.01) and −16.6 ± 1.3 mV (n = 17; p < 0.0005) for I1656M and R1657C, respectively (Fig. 2b). Additionally, both mutants had statistically decreased voltage sensitivity, as suggested by comparison of their slope factors (κ) with that of WT (Table 1). These findings indicate that R1657C and I1656M sodium channels will require a greater degree of membrane depolarization to activate, and this may contribute to altered neuronal excitability.

The two mutants also displayed subtle, yet significant, defects in fast inactivation. I1656M exhibited a slight hyperpolarizing shift in half-maximal steady-state inactivation (V1/2) (Table 1; Fig. 2c), and both mutants had slope factors for inactivation that differed significantly from WT, albeit in opposite directions (Table 1). Recovery from inactivation of WT channels was distributed into two populations, with the majority (83 ± 2%) recovering within a few milliseconds (τ1 = 3.9 ± 0.5 msec) and a minor population of channels (17 ± 2%) recovering more slowly (τ2 = 96 ± 16 msec) (Fig. 2d). In cells expressing R1657C, recovery from fast inactivation was faster in both populations (p < 0.005 and 0.001 for τ1 and τ2, respectively), but there was a significant (p < 0.005) redistribution such that more channels partitioned to the more slowly recovering state (Table 1).

Alterations in slow inactivation

We also tested cells expressing either WT or mutant channels for differences in slow inactivation properties (Fig. 3). In these experiments, the effects of fast inactivation were eliminated by a recovery pulse to −120 mV lasting at least 50 msec (see Materials and Methods). Entry into the slow inactivated state was biphasic, with more than half (57 ± 5%) of WT channels entering with a time constant smaller than 1000 msec (τ1) (Table 2). For R1657C, we observed a significantly (p < 0.05) greater proportion of channels entering the slow inactivated state with the larger time constant (τ2) (Table 2). Steady-state availability after 30 sec depolarizations to various potentials was similar in WT and both mutants, but slow inactivation voltage sensitivity was significantly (p < 0.01) reduced in I1656M (Fig. 3b). When subjected to a 30 sec depolarization, WT channels recovered in a biphasic manner, with a large proportion of the channels (69 ± 2%) repriming quickly (τ1 = 281 ± 45 msec) and the remainder (31 ± 2%) requiring more sustained (τ2 = 4896 ± 1542) hyperpolarization. Under the same conditions, cells expressing R1657C recovered significantly (p < 0.01) faster (τ = 146 ± 17 msec), whereas cells expressing I1656M displayed a significantly (p < 0.05) larger proportion of rapidly recovering channels.
These observations are in keeping with a subtle impairment in slow inactivation for both mutants.

Absence of persistent current in I1656M and R1657C
We previously observed that three other SCN1A mutations associated with GEFS+ including R1648H, also in D4/S4, confer a biophysical defect characterized by persistent, noninactivating whole-cell current. However, neither I1656M nor R1657C generated persistent currents statistically larger than WT as determined by subtraction of TTX-insensitive current (magnitude of persistent current as percentage of peak current amplitude; WT: 0.60 ± 0.07%, n = 7; I1656M: 0.81 ± 0.15%, n = 8; R1657C: 0.84 ± 0.17%, n = 9). This finding was reinforced by the absence of observable channel activation during slow voltage ramps from −120 to +40 mV over 8 sec (data not shown). Therefore, it seems that this single biophysical defect is not shared by all SCN1A mutations associated with GEFS+.

Nonfunctional SCN1A mutations
During this study, we also examined three additional SCN1A mutants associated with either GEFS+ (V1353L and A1685V) (Sugawara et al., 2001a; Wallace et al., 2001b) or SMEI (L986F) (Claes et al., 2001). All three mutations were associated with complete absence of heterologously expressed SCN1A sodium current (peak current amplitude, less than −0.3 nA). We ruled out cloning artifacts as an explanation for nonfunctional sodium channels by verifying the complete coding sequencing of each mutant construct and by examining multiple independent recombinant clones.

The absence of sodium current could have several explanations with primary failure of sodium conductance or defects in protein trafficking representing the two most likely causes. In other ion channel disorders such as cystic fibrosis and congenital long QT syndrome, misfolded proteins can be functionally rescued with a variety of in vitro experimental maneuvers (French et al., 1996; Sato et al., 1996; Rubenstein et al., 1997; Egan et al., 2002; Rajamani et al., 2002; Valdivia et al., 2002). We tested several strategies to rescue the L986F mutation, including lowering the incubation temperature (26°C) of cultured cells after transfection, exposure of cells to sodium channel blockers (phenytoin, lidocaine, or mexiletine) that may stabilize protein conformation, and treatment with thapsigargin to inhibit Ca2+-dependent quality control mechanisms in the endoplasmic reticulum. However, none of these experimental maneuvers were successful in rescuing measurable sodium current. Similarly, low temperature incubation of cells transfected with V1353L or A1685V did not rescue sodium currents.

These findings suggest that nonfunctional alleles may explain either GEFS+ or SMEI and further emphasize that a single biophysical mechanism does not explain epilepsy in these disorders. Our results also suggest that the correlation between in vitro biophysical behavior and clinical syndrome is complex.

Table 1. Biophysical parameters for activation and fast inactivation

<table>
<thead>
<tr>
<th>Voltage dependence of activation</th>
<th>Voltage dependence of fast inactivation</th>
<th>Recovery from fast inactivation</th>
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<tr>
<td>$V_{1/2}$ (mV)</td>
<td>$k$ (mV)</td>
<td>$n$</td>
</tr>
<tr>
<td>WT</td>
<td>-23.6 ± 1.2</td>
<td>7.4 ± 0.3</td>
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<tr>
<td>I1656M</td>
<td>-17.4 ± 1.5$^*$</td>
<td>8.3 ± 0.2$^*$</td>
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<tr>
<td>R1657C</td>
<td>-16.6 ± 1.3$^{**}$</td>
<td>8.3 ± 0.2$^*$</td>
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Values significantly different from WT are marked with asterisks or daggers: *p < 0.05; **p < 0.005; ***p < 0.0005; †p < 0.01; ††p < 0.001; †††p < 0.0001.
Figure 3. Slow inactivation properties of I1656M and R1657C. a, Onset of slow inactivation. Cells were stepped to $-10$ mV for 0.001–100 sec, allowed to recover from fast inactivation at $-120$ mV for 50 msec, and subjected to a $-10$ mV test pulse. b, Steady-state slow inactivation after a 30 sec depolarization to potentials between $-140$ and $-10$ mV. Cells were allowed to recover from fast inactivation at $-120$ mV for 50 msec before the actual test pulse to $-10$ mV. c, Recovery from slow inactivation. Cells were conditioned at $-10$ mV for 30 sec, allowed to recover at $-120$ mV for 0.1–100 sec, and immediately tested at $-10$ mV. Because the intermediate recovery period always exceeded 100 msec, effects of fast inactivation were deemed to be negligible. All data were fitted to a two-exponential or Boltzmann function, as described in Materials and Methods. Fit parameters for all experiments shown are provided in Table 2.

Discussion

The identification of human ion channel mutations in epilepsy has provided new opportunities to investigate the molecular basis of this complex and heterogeneous neurological condition (Meisler et al., 2001). We have focused our efforts on defining the biophysical defects associated with mutations in the human neuronal voltage-gated sodium channel gene SCN1A. Mutations in this gene are associated with GEFS+, often transmitted as an autosomal dominant trait, and SMEI (Dravet syndrome), a more severe disorder with an unclear inheritance pattern (Dravet and Bureau, 1981; Scheffer et al., 2001). In some families, both disorders may be present, suggesting that GEFS+ and SMEI represent a clinical spectrum of the same disease (Singh et al., 2001). More than one hundred SCN1A mutations have been defined to date, with missense mutations being most common in GEFS+ (Escaig et al., 2000, 2001; Abou-Khalil et al., 2001; Sugawara et al., 2001a; Wallace et al., 2001b; Ito et al., 2002) and more deleterious alleles (nonsense, frameshift) representing the majority of SMEI mutations (Claes et al., 2001; Sugawara et al., 2001a; Ohmori et al., 2002; Fujiwara et al., 2003; Nabbout et al., 2003). Given this mutational diversity, it is logical to assume that a range of functional defects account for the varied clinical presentation of these disorders.

Gain or loss of function in GEFS+?

We recently characterized three missense SCN1A mutations associated with GEFS+ (R1648H, T875M, and W1204R) and discovered a shared biophysical defect consistent with a gain of sodium conductance over time because of a persistent, noninactivating current component (Lossin et al., 2002). This functional defect is consistent with the notion of neuronal hyperexcitability attributable to the impact of sustained sodium current on action potential threshold. At least one other human brain sodium channel mutation associated with an inherited epilepsy (R187W in SCN2A) has been demonstrated to exhibit abnormal inactivation leading to increased sodium influx (Sugawara et al., 2001b). Similarly, transgenic mice with neuronal overexpression of an inactivation-impaired Na1.2 sodium channel exhibit severe epilepsy (Kearney et al., 2001). These findings indicate that gain-of-function sodium channel mutations can lead to phenotypes attributed to neuronal hyperexcitability.

In contrast to these previously characterized gain-of-function mutations, we now demonstrate that two mutant SCN1A alleles associated with GEFS+ (I1656M and R1657C) exhibited biophysical features consistent with either reduced function (reduced current density and expression efficiency, depolarizing shift in the voltage dependence of activation) or enhanced function (accelerated recovery from fast and slow inactivation in R1657C), whereas two other missense mutations associated with this disorder (V1353L and A1685V) completely abolished functional sodium channel activity in heterologous cells. Neither I1656M nor R1657C exhibited a noninactivating sodium current component. These data indicate that not all SCN1A mutations...
associated with GEFs+ cause the same biophysical defect and, thus, illustrate functional heterogeneity in this syndrome.

The functional heterogeneity observed for SCN1A mutations may help explain some of the clinical variability in phenotype severity between families segregating different alleles, but it does not explain intrafamilial variation in the clinical expression of the disorder. In the three-generation family in which we discovered R1657C and in many other GEFs+ families, considerable differences in epilepsy severity were evident among mutation carriers, suggesting possible involvement of other genetic or environmental modifiers.

The coexistence of biophysical characteristics with partially offsetting effects as observed for I1656M and R1657C makes simple classification of these alleles as either “gain” or “loss” of function seem inappropriate. Predicting the cellular consequences of these biophysical defects is very difficult, and additional experiments are clearly warranted to define mutation effects in native neurons. Although the subpopulation of neurons most likely affected by these sodium channel disturbances is uncertain, we can speculate that reduced excitability of inhibitory neurons contributes to the seizure susceptibility in this disorder. This notion is consistent with the fact that mutations in a GABA receptor subunit resulting in impaired receptor function cause a similar condition (Baulac et al., 2001; Wallace et al., 2001a; Bianchi et al., 2002). In contrast, subtle impairment of slow inactivation as observed for R1657C may enable more rapid recovery of channels from prolonged membrane depolarizations. This notion, however, disagrees with findings of other groups that have observed enhanced susceptibility to slow inactivation with different GEFs+-associated SCN1A mutants (Alekov et al., 2000, 2001; Spamanato et al., 2001). Experimental variations, such as the use of a mammalian expression system in our study or use of the human SCN1A versus SCN4A (Alekov et al., 2000, 2001) or rat Scn1A (Spamanato et al., 2001) may account, in part, for these observed differences. Alternatively, the observed differences may reflect the actual biophysical heterogeneity among SCN1A mutants. The expression pattern of SCN1A is broad, and specific localization in inhibitory neurons is not known (Whitaker et al., 2000). Careful analysis of the spatial and temporal distribution of SCN1A, its splice isoforms, and their regulation is expected to improve our understanding of these disorders as will development of animal models expressing GEFs+ mutations in defined brain regions.

Functional SCN1A haploinsufficiency in SMEI and GEFs+

The majority of SCN1A mutations found to date in SMEI have been de novo nonsense or frameshift alleles that are presumed to encode nonfunctional sodium channels (Claes et al., 2001; Sugawara et al., 2001a; Ohmori et al., 2002; Nabbout et al., 2003). We characterized one missense mutation (L986F) associated with SMEI from prolonged membrane depolarizations. This notion, however, disagrees with findings of other groups that have observed enhanced susceptibility to slow inactivation with different GEFs+-associated SCN1A mutants (Alekov et al., 2000, 2001; Spamanato et al., 2001). Experimental variations, such as the use of a mammalian expression system in our study or use of the human SCN1A versus SCN4A (Alekov et al., 2000, 2001) or rat Scn1A (Spamanato et al., 2001) may account, in part, for these observed differences. Alternatively, the observed differences may reflect the actual biophysical heterogeneity among SCN1A mutants. The expression pattern of SCN1A is broad, and specific localization in inhibitory neurons is not known (Whitaker et al., 2000). Careful analysis of the spatial and temporal distribution of SCN1A, its splice isoforms, and their regulation is expected to improve our understanding of these disorders as will development of animal models expressing GEFs+ mutations in defined brain regions.

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