Gating Properties of *SCN5A* Mutations and the Response to Mexiletine in Long-QT Syndrome Type 3 Patients

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Background—Mexiletine (Mex) has been proposed as a gene-specific therapy for patients with long-QT syndrome type 3 (LQT3) caused by mutations in the cardiac sodium channel gene (*SCN5A*). The degree of QT shortening and the protection from arrhythmias vary among patients harboring different mutations. We tested whether the clinical response to Mex in LQT3 could be predicted by the biophysical properties of the different mutations.

Methods and Results—We identified 4 *SCN5A* mutations in 5 symptomatic LQT3 patients with different responses to Mex (6 to 8 mg · kg^{-1} · d^{-1}). We classified the mutations as sensitive to Mex (P1332L, R1626P; $\geq$10% of QTc shortening and QTc <500 ms or no arrhythmias) or insensitive to Mex (S941N, M1652R; negligible or no QTc shortening and sudden death). We measured Na$^+$ current from HEK 293 cells transfected with wild-type (WT) or mutant Nav1.5. All mutations showed impaired inactivation of Na$^+$ current, but the mutations identified in patient responders to Mex (P1332L, R1626P) showed a hyperpolarizing shift of $V_{1/2}$ of steady-state inactivation. Furthermore, Mex produced use-dependent block with the order R1626P=P1332L>S941N=WT>M1652R, suggesting that Mex-sensitive mutants present prolonged recovery from Mex block.

Conclusions—We propose that voltage dependence of channel availability and shifts of $V_{1/2}$ of steady-state inactivation correlate with the clinical response observed in LQT3 patients. This supports the view that the response to Mex is mutation specific and that in vitro testing may help to predict the response to therapy in LQT3. (*Circulation*. 2007;116:1137-1144.)

Key Words: electrophysiology ■ genetics ■ long-QT syndrome ■ pharmacology ■ sodium ■ ion channels

The congenital long-QT syndrome type 3 (LQT3) is caused by mutations in the *SCN5A* gene encoding the α subunit of the human cardiac voltage-gated sodium channel hNav1.5, which is characterized by an abnormally prolonged QT interval and by life-threatening arrhythmias. LQT3 mutations produce a gain of function, most of them impairing fast inactivation so that the decay of the current occurs more slowly or not completely, thus leading to QT interval prolongation.[1]

Clinical Perspective p 1144

On the basis of encouraging clinical and experimental investigations,[2,3] it has been suggested that mexiletine (Mex) could shorten QT interval in LQT3 patients by attenuating the increase in the I_{Na} that is associated with the presence of *SCN5A* mutations. Clinical evidence has shown that although Mex causes a pronounced QT shortening in some LQT3 patients, in others the effect of the drug is much less pronounced and QTc duration remains >500 ms, a value associated with higher risk of arrhythmic events.[4] Even more important, not all patients are protected from the development of

life-threatening arrhythmias by Mex despite compliance with treatment. For example, in a child carrier of the S941N *SCN5A* mutation followed up at our center, QTc shortened after Mex but remained >500 ms,[5] and the child died suddenly at 5 years of age while on treatment with β-blockers and Mex.

It is currently unknown whether the extent of QTc shortening (QTcSH) during Mex treatment is at least partially dependent on the type of *SCN5A* mutation carried by the patients. The answer to this question is very important for the management of patients because it could allow prediction of the clinical efficacy of Mex treatment in patients. In the present study, we tested the hypothesis that the clinical response to Mex is influenced by the biophysical properties of the mutations.

Methods

We identified in our LQTS clinical database 5 LQT3 probands followed up at our LQTS clinics with documented ventricular tachyarrhythmias before therapy who were treated with Mex for at least 12 months, regularly attended follow-up visits at our clinics, and showed compliance with the drug regimen.
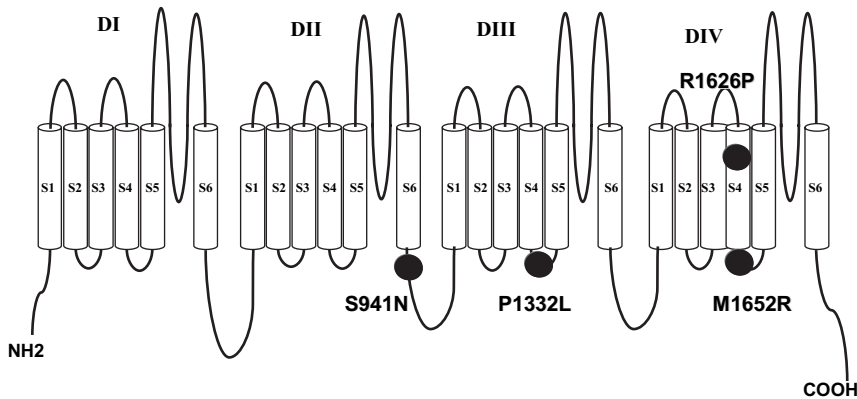
Figure 1. Predicted topology of the α subunit of the Nav1.5 cardiac sodium channel and localization of the 4 LQT3 mutations described.

Clinical Evaluation and Follow-Up

LQTS was diagnosed on the basis of standard clinical criteria.[6] ECG parameters were measured manually on at least 4 ECG recordings for each patient before and during Mex (6 to 8 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}) therapy. QT was measured in limb lead II on 3 consecutive beats and corrected for heart rate (Bazett's formula).

Molecular Screening

Genetic analysis was performed as previously reported.[4] In all patients, screening of the open reading frame of the *SCN5A*, *KCNH2*, *KCNQ1*, *KCNE1*, and *KCNE2* genes was performed.

Site-Directed Mutagenesis and Transfection in HEK Cells

The *SCN5A* mutations were engineered into wild-type (WT) cDNA cloned in pcDNA3.1 (Invitrogen, Carlsbad, Calif) and confirmed by sequence analysis. HEK 293 cells were transfected with equal amount of Na$^+$ channel α subunit and hβ_1 by a lipofection procedure as previously described.[7]

Electrophysiology

Membrane currents were measured using whole-cell patch clamp procedures with Axopatch 200B amplifiers (Axon Instruments, Foster City, Calif). Internal pipette solution contained (mmol/L) aspartic acid 50, CsCl 60, Na$_2$-ATP 5, EGTA 11, HEPES 10, CaCl$_2$ 1, and MgCl$_2$ 1, with pH 7.4 adjusted with CsOH. External solutions (full Na$^+$) consisted of (mmol/L) NaCl 130, CaCl$_2$ 2, CsCl 5, MgCl$_2$ 1.2, HEPES 10, and glucose 5, with pH 7.4 adjusted with CsOH. In experiments designed to measure the voltage dependence of activation, external Na$^+$ was reduced to 30 mmol/L with *N*-methyl-glucamine used as a Na$^+$ substitute. Recordings were made at room temperature. Holding potentials were -100 mV unless otherwise indicated. Sustained sodium current (I$_{sus}$) was measured 150 ms after depolarization to -10 mV and determined by subtracting background currents measured in the presence of tetrodotoxin (30 μmol/L, Sigma Chemical Co, St Louis, Mo) from tetrodotoxin-free records. Steady-state inactivation was determined after application of conditioning pulses (500 ms for control, 5 seconds for Mex) applied to a series of voltages with an interpulse interval of 5 seconds for control and 30 seconds for Mex. Steady-state activation was esti-

mated by measuring peak sodium current during a variable test potential. Current at each membrane potential was divided by the electrochemical driving force for sodium ions and normalized to the maximum sodium conductance. Data for the voltage dependence of activation and inactivation were fitted with the Boltzmann equation. Recovery from inactivation and drug block was measured in paired pulse experiments. Details of each pulse protocol are given schematically in the figures. Data for the time course of recovery were fitted with functions of 2 exponentials. Tonic block (TB) was measured at 0.033 Hz after steady state was achieved in the presence of Mex. Steady-state use-dependent block (UDB) was reached in response to a train of 50 pulses, with a protocol that mimics the action potential duration and tachycardia arrhythmia, ie, depolarize to -10 mV for 400 ms at a rate of 120 per minute. UDB was measured as block induced by pulse trains relative to TB for a given drug concentration. Concentration-response curves were fitted with the following equation: $I/I_{max} = 1/[1 + (drug)/EC_{50}]^n$.

Statistical Analysis

Pclamp9.2 (Axon Instruments) and Excel (Microsoft, Seattle, Wash) were used for data acquisition and analysis. Data are presented as mean$\pm$SE. An unpaired Student *t* test and 1-way ANOVA followed by a Tukey test were used to compare means. Values of $P<0.05$ were considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Characterization of the Response to Mex in LQT3 Patients

All 5 LQT3 patients were symptomatic for syncope and/or documented ventricular arrhythmias before initiation of Mex treatment. All were carriers of *SCN5A* mutations (Figure 1) and were treated with oral Mex (6 to 8 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}) in addition to β-blocker therapy (nadolol 1 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}). The clinical characteristics of the 5 patients are reported in Table 1. As shown in Figure 2, 3 patients responded to Mex with a

TABLE 1.   Clinical Characteristics of LQT3 Patients

Patients	Mutation	Age at Last Follow-Up, y	Symptoms Before Therapy	Before Mex, QTc, ms/HR, bpm	After Mex, QTc, ms/HR, bpm	QTcSH, ms (%)	Events on Mex (Follow-Up, y)
1	R1626P	20.5	TdP	480/80	420/74	60 (12.5)	Alive, no cardiac events (7)
2	P1332L	8.25	TdP	570/100	493/95	77 (13.5)	Alive, no cardiac events (4)
3	P1332L	11	TdP	506/125	455/107	51 (10)	Alive, no cardiac events (6)
4	S941N	5	TdP	600/107	570/80	30 (5.0)	SCD (VF) (5)
5	M1652R	1	TdP	607/75*	607/100	0 (0)	SCD (VF) (1)

TdP indicates torsade de pointes; SCD, sudden cardiac death; and VF, ventricular fibrillation.

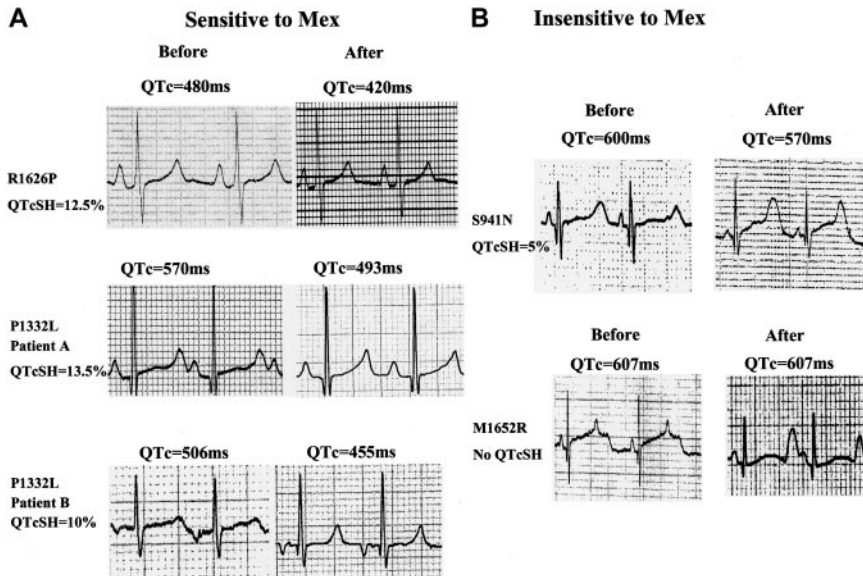*2:1 atrioventricular block.

Figure 2. ECG of LQT3 patients before and after Mex therapy. A, R1626P and P1332L are defined as being sensitive to Mex therapy (see text for definition). B, S941N and M1652R are defined as being insensitive to Mex (see text for definition)

QTcSH, whereas the other 2 had negligible or no QTcSH. The parents of the 2 patients with poor response to Mex refused an implantable cardioverter-defibrillator for their children. Over a mean follow-up of 4.6 years on Mex in the 5 patients, 3 subjects remained free of cardiac events (mean follow-up, 5.7 years on Mex). Two patients died on cardiac arrest during Mex therapy (after 1 and 5 years of follow-up, respectively).

According to outcome data and the extent of QTcSH after Mex, the 4 mutations were defined as sensitive to Mex (P1332L, R1626P; QTcSH >10%, QTc <500 ms, and arrhythmia-free survival) and insensitive to Mex mutations (S941N, M1652R; negligible QTcSH and sudden cardiac death).

Biophysical Profile of Mutations

Time Course of Inactivation and Sustained Sodium Current

Whole-cell current recordings from cells expressing WT, P1332L, R1626P, S941N, or M1652R are shown in Figure 3. All clones exhibited rapid activation and inactivation in response to a series of depolarizing test potentials typical of voltage-gated sodium channels. The most remarkable biophysical characteristic of P1332L, R1626P, S941N, and M1652R compared with WT was the presence of delayed onset of inactivation (Figure 4A). In 3 mutations (P1332L, S941N, and M1652R), the delay of inactivation was mild; the fourth mutant clone (R1626P) exhibited a more severe impairment of the kinetic of onset of inactivation that showed almost no voltage dependency (Figure 4A).

Because increased I_{sus} is a common dysfunction associated with LQT3 mutations,[1] we performed experiments targeted to assess whether sustained Na$^+$ activity was present in the mutations identified in our patients (Figure 4B). Three of the 4 mutations (R1626P, S941N, M1625R) presented a larger I_{sus} than WT, whereas the P1332L current showed no augmented I_{sus} compared with WT. Overall, it is concluded that the presence of increased I_{sus} is not necessary for the LQT3 phenotype and that the amplitude of the I_{sus} does not allow discrimination between mutations that are Mex sensitive and those that are Mex insensitive.
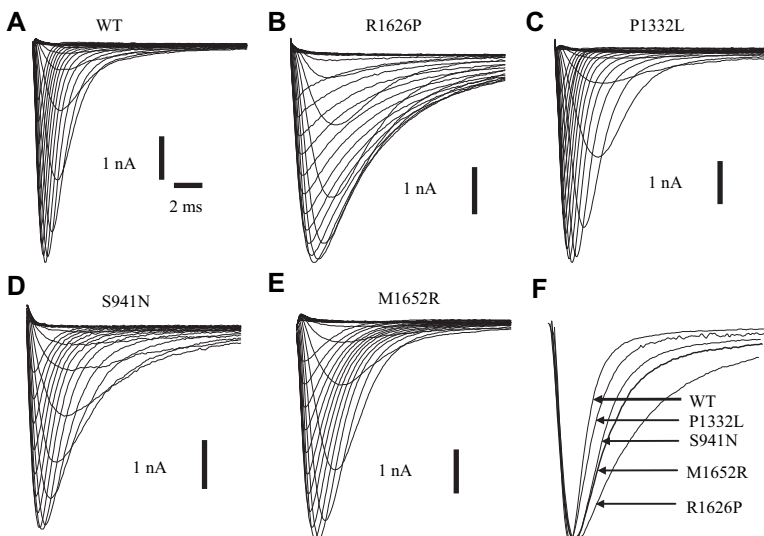
Figure 3. Whole-cell current recordings of WT and mutant *SCN5A* channels. A through E, Na channels were expressed by transient transfection in HEK cells in the presence of hβ1 and currents recorded at various membrane potentials from −80 to 30 mV in 5-mV increments from a holding potential of −100 mV. F, Normalized Na$^+$ current at a test potential of −20 mV for WT, R1626P, P1332L, S941N, and M1652R.
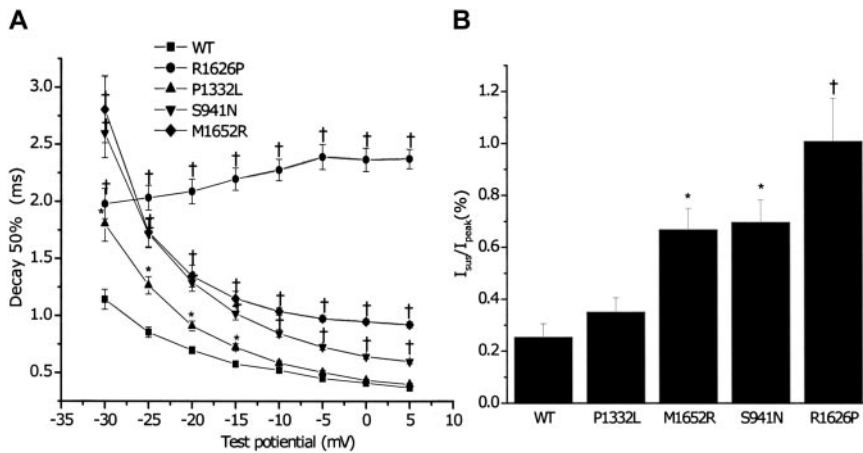
Figure 4. A, Time to half inactivation (measured as time to 50% decay of transient inward current) during pulse plotted vs test pulse voltage. Statistically significant differences comparing mutant channels with WT are shown (*$P<0.05$, †$P<0.001$, Student t test). n=6 to 9 cells. B, The amplitudes of I_{sus} (I_{sus}/I_{peak}%) in WT, R1626P, P1332L, S941N, and M1652R were 0.25 ± 0.05, 1.01 ± 0.17, 0.35 ± 0.06, 0.70 ± 0.09, and 0.67 ± 0.08, respectively. n=7 to 21 cells. *$P<0.05$, †$P<0.001$ (comparisons with WT using 1-way ANOVA followed by a Tukey test).

Steady-State Inactivation and Activation

The S941N mutation showed no differences in the voltage dependency of inactivation compared with WT, whereas M1652R showed a curve shifted toward positive potentials by 7 mV (rightward shift in Figure 5A); on the contrary, R1626P and P1332L (ie, the 2 mutations present in the patients with a positive response to Mex) showed steady-state inactivation curves shifted in the negative direction by 7 and 6 mV, respectively (leftward shift in Figure 5A). The slope factor for R1626P was increased to 9.49 mV compared with the value of ≈5 to 6 mV observed in the WT and in the other mutants (S941N, P1332L, M1652R). No significant differences in steady-state activation were observed between WT and 3 of the mutants (R1626P, S941N, M1652), whereas steady-state activation of P1332L was shifted toward negative potentials by 5 mV (Figure 5B). Overall, we conclude that the 2 Mex-sensitive mutations presented a negative shift of the steady-state inactivation curves.

Recovery From Inactivation

Previous data showed that some LQT3 mutations speed the rate of recovery of the channel from inactivation, thus enhancing the gain-of-function behavior of the mutant channel compared with WT.[7] We investigated recovery from inactivation in WT and mutant channels. Figure 5C shows that M1652R recovers faster than WT, that P1332L recovers more slowly than WT, and that S941N and R1626P show a recovery from inactivation that is not different from that observed in WT. We conclude that although faster recovery from inactivation contributes to the gain of function in LQT3, not all LQT3-causing mutations present this behavior, and in our series, the kinetics of recovery from inactivation is not different between Mex-sensitive and -insensitive mutants.

Effects of Mex on TB and UDB

We tested whether mutations identified in patients with a clinically positive response to Mex had different binding kinetics compared with the mutations identified in patients refractory to the drug. Accordingly, we characterized the TB and UDB of Mex in WT and each of the 4 mutants.

As shown in Figure 6B, R1626P presented the greatest and M1652R presented the smallest TB, with EC_{50} values of 153 and 944 μmol/L, respectively. However, we cannot conclude that the difference in the response to Mex observed in patients with the 4 mutations under study can be accounted for by the TB for 2 reasons: no difference in the EC_{50} of P1332L and S941N can be observed despite the remarkably different response to the drug in the carriers of the 2 mutations, and over the range of drug concentrations relevant in a clinical
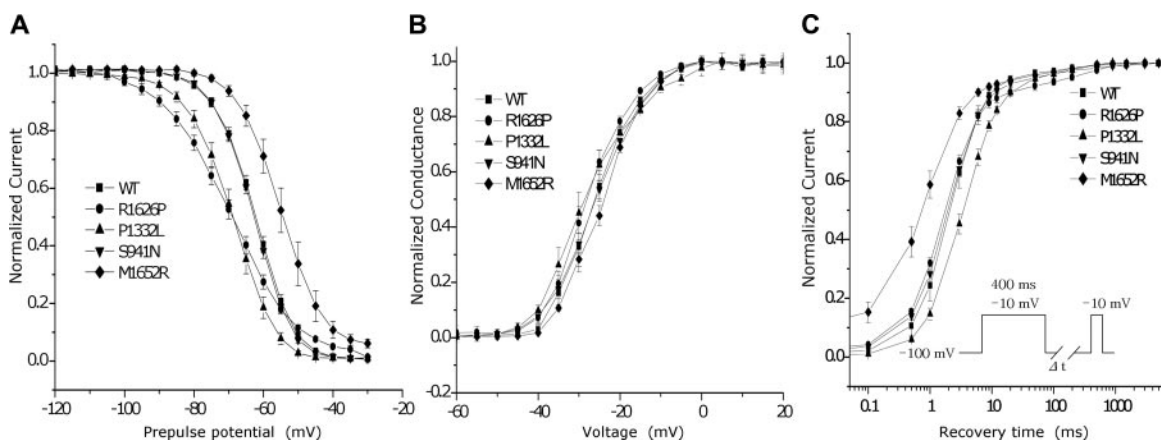

Figure 5. Voltage dependence of inactivation (A) and activation (B) for WT, R1626P, P1332L, S941N, and M1652R sodium channel. Experimental data were fitted with Boltzmann relationships to obtain the parameters shown in Table 2. C, Recovery from inactivation (protocol in inset) was fitted using a biexponential function. Time constants and relative weights on averaged data are shown in Table 2.
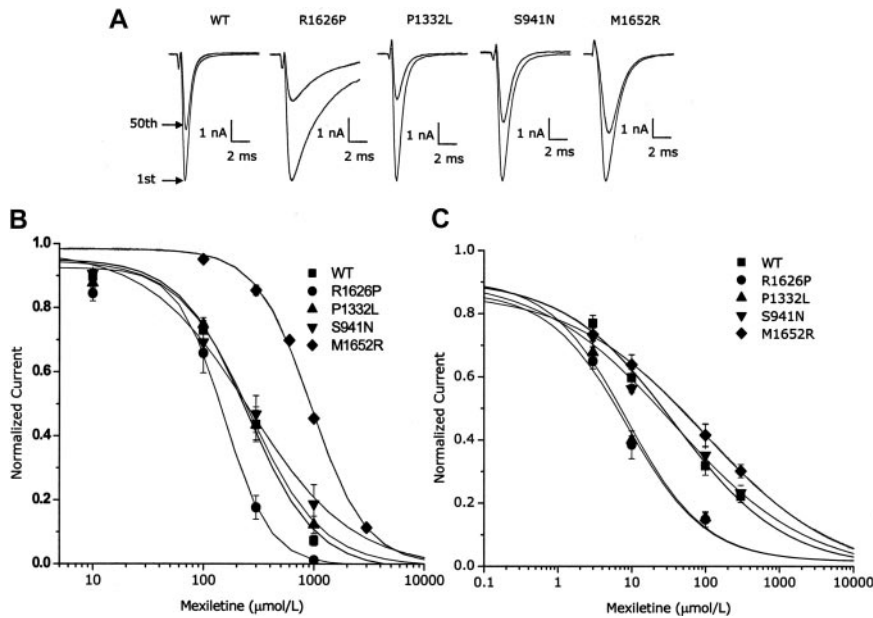
Figure 6. A, UDB measurement for each construct and superimposition of current in the presence of 10 μmol/L Mex. B, Concentration dependence of TB by Mex. Graph shows peak current after drug application normalized to peak current in absence of drug plotted as a function of drug concentration. EC_{50} values were 253 μmol/L for WT, 153 μmol/L for R1626P, 263 μmol/L for P1332L, 260 μmol/L for S941N, and 944 μmol/L for M1652R. n=4 to 10 cells per condition. C, Concentration dependence of UDB by Mex. EC_{50} values were 37.99 μmol/L for WT, 8.8 μmol/L for R1626P, 8.76 μmol/L for P1332L, 37.57 μmol/L for S941N, and 96.14 μmol/L for M1652R. n=4 to 10 cells per condition.

setting (ie, in the range of 10 μmol/L[8]), no difference in the TB was observed among the mutations.

Interestingly, at a clinically relevant concentration of 10 μmol/L (Figure 6A), we observed differences in the UDB of WT, R1626P, P1332L, S941N, and M1652R (40.2±2.6%, 61.5±4.4%, 60.0±1.4%, 43.7±4.0%, and 36.2±3.2%). When we compared UDB between Mex-sensitive and -insensitive mutations, we found a highly significant difference ($P<0.001$, 1-way ANOVA followed by a Tukey test). This difference was evident over a broad range of concentrations. Thus, the EC_{50} for Mex UDB of R1626P and P1332L channels is roughly 4 times lower than the EC_{50} for WT and S941N and 10 times lower than that for block of M1652R (Figure 6C).

Recovery From Mex Block
To further characterize the greater UDB observed in the 2 Mex-sensitive mutations, R1626P and P1332L, we characterized the recovery time course from Mex block at a clinically relevant concentration (10 μmol/L),[8] which is shown in Figure 7A. Figure 7B shows enlargement of the slow phase of recovery; A_{slow} and τ_{slow} represent the recovery from drug block[9] that is

increased in R1626P and P1332L mutant channels. These results suggest that Mex blocks R1626P and P1332L channels more efficiently than the WT and the other 2 mutant channels.

Affinity of Mex to the Resting State and Inactivated State
As described by Bean et al,[10] the apparent dissociation constant of Mex for the resting state channel (K_R) was estimated directly by applying Mex at a very negative holding potential when all channels are in the resting state. We determined the EC_{50} at increasingly negative holding potentials, with the more negative potential being set at −140 mV. EC_{50} values were 786, 663, 734, 884, and 932 μmol/L for WT, R1626P, P1332L, S941N, and M1652R, respectively. Although the EC_{50} tended to differ in the same way as at −100 mV, the differences were much smaller and appeared to be converging toward a common value at $\approx$1000 μmol/L. These results suggest that, although TB is different for the 5 channels, the actual K_R may not be different but rather reflects the difference of TB at a holding potential that is "contaminated" by inactivated state.
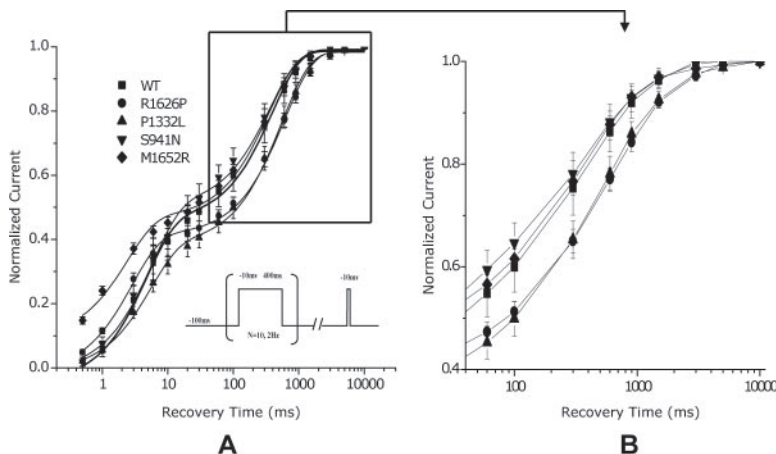

Figure 7. A, Effect of mutations on time course of recovery from inactivation and 10 μmol/L Mex block (protocol in inset). Normalized current is plotted against recovery interval. n=4 to 6 cells per condition. B, Enlargement of the slow phase of recovery. Time constants and relative weights on averaged data were as follows: for WT, τ_{fast}=4.7 ms, A_{fast}=0.50, τ_{slow}=393.6 ms, and A_{slow}=0.51; for R1626P, τ_{fast}=2.8 ms, A_{fast}=0.42, τ_{slow}=613.5 ms, and A_{slow}=0.57; for P1332L, τ_{fast}=5.3 ms, A_{fast}=0.39, τ_{slow}=545.8 ms, and A_{slow}=0.61; for S941N, τ_{fast}=5.7 ms, A_{fast}=0.51, τ_{slow}=365.8 ms, and A_{slow}=0.49; for M1652R, τ_{fast}=2.4 ms, A_{fast}=0.48, τ_{slow}=350.4 ms, and A_{slow}=0.52.

TABLE 2. Biophysical Properties of *SCN5A* Mutations

	Steady-State Availability			Voltage Dependence of Activation			Recovery From Inactivation		
	$V_{1/2}$, mV	k, mV	n	$V_{1/2}$, mV	k, mV	n	τ_{fast}, ms (%)	τ_{slow}, ms (%)	n
WT	−62.5±0.68	−5.6±0.15	10	−23.2±0.86	5.7±0.37	5	2.9 (94)	101 (6)	11
R1626P	−69.6±1.27*	−9.49±0.15†	6	−26.3±0.79	6.0±0.41	5	2.3 (88)	184 (12)	7
P1332L	−68.9±1.26†	−5.35±0.14	8	−28.2±0.87†	7.4±0.94	5	4.4 (91)	94 (10)	8
S941N	−62.8±0.58	−5.5±0.21	7	−23.8±0.63	6.8±0.21	6	2.6 (91)	96.8 (8)	7
M1652R	−54.9±1.7†	−5.26±0.14	8	−22.5±0.50	5.2±0.09	5	1.1 (91)	54.6 (9)	6

Because the functions for recovery from inactivation were fitted to the averaged data, error estimates on these parameters were not obtained.

*$P<0.05$, †$P<0.01$ (comparisons with WT using 1-way ANOVA followed by a Tukey test).

The apparent dissociation constant for the inactivated state (K_I) was estimated according to the equation of Bean et al[10]: $\Delta V_{1/2}=k\ln[(1+C/K_R)/(1+C/K_I)]$, where $\Delta V_{1/2}$ is the shift of $V_{1/2}$ of steady-state inactivation induced by Mex, k is the slope factor of the steady-state inactivation curve, and C is the concentration of Mex. The K_Is of WT, R1626P, P1332L, S941N, and M1652R were 13.1, 13.8, 11.2, 12, and 12.1 μmol/L, respectively.

According to the modulated-receptor hypothesis, the apparent affinity for Mex ($1/K_{app}$) will depend strongly on the proportion of channels in the resting and inactivated states (comprising factions h and 1−h, respectively). At equilibrium, $1/K_{app}=h/K_R+(1-h)/K_I$. Steady-state inactivation curves represent the voltage-dependent distribution between the resting state and the inactivated state.[10] From our results, the affinities for resting and inactivated channels ($1/K_R$ and $1/K_I$, respectively) are quite similar among mutations and therefore unlikely to account for the different responses to the drug observed in the carriers.

Discussion

Gene-Specific Therapy for LQT3?

LQTS is an inherited arrhythmogenic disease characterized by prolongation of QT interval and susceptibility to ventricular tachyarrhythmias in response to sympathetic activation. Among the several genetic subtypes of this disease, the uncommon form associated with mutations in the cardiac sodium channel gene *SCN5A* presents phenotypic manifestations that are unique. At variance with the other forms of LQTS, LQT3 patients predominantly experience cardiac events at rest and not during exercise or emotion,[11] are poor responders to β-blocker therapy,[12] and have a rather adverse prognosis in the absence of treatment.[4] Accordingly, the management of LQT3 patients is rather complex, and an implantable cardioverter-defibrillator is often the choice to reduce the risk of sudden cardiac death in these patients.[12] Since the early in vitro characterization of the biophysical properties of LQT3-related mutations identified in the *SCN5A* gene,[1] it became clear that the fundamental abnormality in LQT3 is represented by an excess of inward sodium current that may be caused by delayed inactivation or by the presence of I_{sus}. As a consequence, use of sodium channel blocking agents as a "gene-specific therapy" for LQT3 progressively emerged and was tested in the clinical setting.[2,13] However, the role of sodium channel blockers is still poorly defined. Given that in the medical literature negative results are often underrepresented,[14] more reports exist of the efficacy of sodium channel blockers in LQT3 patients than reports of the failure of the same drugs to protect patients. However, investigators with large referral of LQTS patients have consistently noticed that not all patients respond to these drugs with a comparable degree of QT interval shortening and that in some LQT3 patients sodium channel blockers do not prevent life-threatening arrhythmias. In the present study, we investigated whether the response to Mex in LQT3 patients could be inferred by the in vitro functional profile of the mutant sodium channels.

SCN5A Mutations Present in LQT3 Patients With Long-Term Mex Therapy

We identified 5 LQT3 probands who were treated for an average of 4.6 years with oral Mex who presented variable degree of shortening of the QT interval in response to the drug. The 5 patients carried 4 different mutations that were localized in different regions of the predicted topology of the protein (Figure 1).

The R1626P mutant is located in DIV/S4 of the channel, which acts as a voltage sensor and moves in response to changes in the membrane potential. This movement is disturbed by the neutralization of positive charges such as when an arginine is replaced with cysteine, glutamine, proline, or histidine. It has been reported that mutations in this region usually cause a hyperpolarizing shift of the steady-state inactivation curve that makes the mutant sodium channel more sensitive to sodium channel blockade.[9,15,16]

The P1332L mutant is located in the DIII/S4-S5 (Figure 1) linker of the channel. At variance with the other LQT3 mutations studied in the present study, P1332L was the only one devoid of I_{sus}. Interestingly, a lack of I_{sus} has been reported in other *SCN5A* mutations located in close proximity to P1332L (ie, A1330T and A1330P).[17] When expressed in HEK cells, P1332L showed a delayed time course of current decay that is consistent with the association with the LQTS phenotype of the carriers. Interestingly, 2 unrelated individuals (probands) carried this mutation, and both showed a remarkable shortening of the QT interval, suggesting that the effect is likely to be mutation specific. To support this view, it is worth noting that this very same mutation has been identified by another team of investigators[18,19] in an LQT3 patient who also showed a remarkable response to Mex administration.

The M1652R mutant is located in the DIV/S4-S5 (Figure 1) linker of the channel and is the first reported and characterized *SCN5A* mutation in this segment. The fact that

mutations of *SCN4A* in this region impair the fast inactivation suggests that this segment plays an important role in the fast inactivation of sodium channel.[20] In vitro characteristics of M1652R support this view and are consistent with the LQTS phenotype of the carrier.

The S941N mutant is located in the DII-DIII linker (Figure 1) of the channel. Previously, we characterized S941N mutation in oocyte expression system and demonstrated that this mutant exhibited delayed inactivation and the presence of I_{sus}.[5] In the present study, we confirmed the same findings, expressing the mutation in HEK cells.

All 4 *SCN5A* mutations caused gain of function that is consistent with the phenotype of LQTS; however, as demonstrated by other authors in previous studies, the severity of channel dysfunction assessed in vitro is not correlated with the prolongation of QT interval observed in patients.[21]

Biophysical Properties of *SCN5A* Mutations and Clinical Efficacy of Mex
We extensively characterized the biophysical properties of the mutant channels identified in LQT3 probands with different responses to oral Mex in the attempt to determine whether we could identify properties of mutant channels that account for the response to Mex of the carriers.

Gating of SCN5A *Mutants Defines the Response to Mex*
Drug-binding affinity to gating state of sodium channel plays an important role in the drug-target interaction. In the *SCN5A* channel, the local binding site of the anesthetic is represented by Phe1760 and Tyr1767[22]; mutations located in close proximity to this binding site such as the S1710L *SCN5A* mutant impair the binding of drugs and manifest faster recovery from block.[23] None of our mutations, however, are located close to the binding site to suggest that this mechanism could influence the response to the drug observed in the clinic.

It is known that the efficacy of sodium channel blockers depends on the gating state of sodium channel. Several models have been proposed to explain the complex interactions between the gating state of sodium channels and the binding of local anesthetics. We therefore speculated that a change in the gating properties of the mutant channels could alter the efficacy of the drug.

It is known that Mex binds to the inactivated state of the sodium channel.[16] During the cardiac cycle, the membrane voltage changes between the resting potential and the plateau voltage, so the proportion of sodium channels in the inactivated state changes with the membrane potential. Previous studies showed that the time course of drug binding occurs over a time scale between several hundreds of milliseconds to several seconds.[8] Therefore, repetitive stimulation can cause accumulation of the block of the sodium channels. It is therefore reasonable to believe that if a mutation in the *SCN5A* gene modifies the properties of the channel in a way that favors the inactivated state, then the channel may be more prone to bind Mex and therefore the drug may be clinically more effective.

Accordingly, in the mutations identified in patients responding to Mex with a shortening of the QT interval of at least 10%, we found a hyperpolarizing shift of $V_{1/2}$ of steady-state inacti-

vation that indicates an increased persistence of the sodium channel in the inactivated state during the cardiac cycle.

UDB of Peak Current Parallels the Clinical Efficacy of Mex
Kambouris et al[15] reported data showing that enhancement of an already altered closed-state inactivation explains the unusual sensitivity of the mutant R1623Q channel to lidocaine, a class Ib drug like Mex. The same authors performed modeling experiments supporting the view that other mutations would share the same response to Mex or lidocaine and proposed that the drug effect on closed-state inactivation may be the key mechanism by which sodium channel blockers exert their beneficial effect in LQT3. These authors came to these conclusions investigating the TB. In our study, however, we could not explain the response to Mex by differences in the TB because, although closed-state inactivation could be advocated to account for the higher sensitivity to Mex of the R1626P mutant, it could not account for the higher sensitivity observed for the other mutation identified in patients who responded to Mex, ie, P1332L. We therefore investigated the TB and UDB of peak current in these 4 *SCN5A* mutations in the presence of Mex using a protocol that mimics the action potential duration of human cardiac myocytes. As expected, the EC_{50} of TB was significantly higher than the EC_{50} of UDB and was much higher than the range of concentrations[8] encountered in clinical settings, making the extrapolation of results to the clinic quite difficult. Interestingly, however, UDB investigated in vitro paralleled the clinical efficacy of Mex in the 4 LQT3 mutations under investigation with EC_{50} well within the range of the plasma levels observed in clinical settings. Furthermore, the 2 mutations identified in the 3 patient responders to the drug were 4- to 10-fold lower than those observed in the mutations carried by patients presenting a more modest QT interval shortening who died suddenly on drug treatment.

Critical Considerations
We report on a limited number of patients; however, considering that LQT3 patients represent no more than 10% to 12% of clinically affected LQTS patients and that not all of them manifest documented torsade de pointes, it is clear that it is not easy to expand these observations. We also recognize that other factors such as compliance to therapy, pharmacokinetic and metabolic factors, activity of Mex on other ionic currents, and interaction between different electrophysiological properties of each mutation are important determinants of the response to therapy. These factors, alone or in combination, may account for the variable response to therapy that is often encountered, even among family members who carry the same mutation. We believe, however, that our data provide a template for selecting management strategies for LQT3 patients, who are the most difficult LQTS patients to treat.

Conclusion
The objective of the present investigation was to assess whether the shortening of the QT interval and efficacy of Mex in preventing ventricular tachyarrhythmia could be predicted on the basis of the profile of the specific *SCN5A* mutations. Our results suggest that abnormalities of the steady-state inactivation leading to a more negative $V_{1/2}$ and the assess-

ment of the EC_{50} for the UDB allow us to speculate that it may be possible to predict the therapeutic effect of Mex beyond the measurement of the QT interval.

We suggest that this scheme for in vitro assessment of Mex effect on *SCN5A* mutations should now be tested in a prospective study that combines long-term follow-up of patients and in vitro characterization of their mutations.

Sources of Funding

Disclosures

None.

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CLINICAL PERSPECTIVE

Long-QT syndrome type 3 (LQT3) is caused by mutations in the sodium channel gene (*SCN5A*) that increase sodium current and prolong cardiac repolarization. LQT3 patients are more likely to have cardiac arrhythmias at rest and incomplete protection when treated with β-blockers; consequently, LQT3 patients have a more adverse prognosis than most LQTS patients. The sodium channel blocker mexiletine has been proposed as a treatment in LQT3 patients to reduce sodium inward current and to shorten QT interval. Clinical data demonstrate that mexiletine does not abbreviate repolarization in all LQT3 patients; the cause for such a variable response is unknown. We hypothesized that the response to mexiletine could be at least partially inferred from in vitro assessment of the functional profile of the different mutations found in patients. Therefore, we studied in vitro mutations found in patients with variable responses to mexiletine. Our data showed that patients who have a shortened QT interval and a favorable prognosis in response to mexiletine carry mutations that shift the steady-state inactivation curves to negative voltages and cause a higher use-dependent block of mexiletine. On the contrary, patients whose QT intervals are not modified in response to mexiletine carry mutations that do not shift the steady-state inactivation curve or shift it to positive voltages. We propose that if these data are confirmed in a larger and prospective study, it will be reasonable to characterize *SCN5A* mutations in vitro to predict the response to mexiletine and to tailor treatment for LQT3 patients.

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Gating Properties of *SCN5A* Mutations and the Response to Mexiletine in Long-QT Syndrome Type 3 Patients

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