Molecular Pharmacology of the Sodium Channel Mutation D1790G Linked to the Long-QT Syndrome

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Background—Multiple mutations of SCN5A, the gene that encodes the human Na+ channel α-subunit, are linked to 1 form of the congenital long-QT syndrome (LQT-3). D1790G (DG), an LQT-3 mutation of the C-terminal region of the Na+ channel α-subunit, alters steady-state inactivation of expressed channels but does not promote sustained Na+ channel activity. Recently, flecainide, but not lidocaine, has been found to correct the disease phenotype, delayed ventricular repolarization, in DG carriers.

Methods and Results—To understand the molecular basis of this difference, we studied both drugs using wild-type (WT) and mutant Na+ channels expressed in HEK 293 cells. The DG mutation conferred a higher sensitivity to lidocaine (EC50, WT = 894 and DG = 205 μmol/L) but not flecainide tonic block in a concentration range that is not clinically relevant. In contrast, in a concentration range that is therapeutically relevant, DG channels are blocked selectively by flecainide (EC50, WT = 11.0 and DG = 1.7 μmol/L), but not lidocaine (EC50, WT = 318.0 and DG = 176 μmol/L) during repetitive stimulation.

Conclusions—These results (1) demonstrate that the DG mutation confers a unique pharmacological response on expressed channels; (2) suggest that flecainide use-dependent block of DG channels underlies its therapeutic effects in carriers of this gene mutation; and (3) suggest a role of the Na+ channel α-subunit C-terminus in the flecainide/channel interaction. (Circulation. 2000;102:921-925.)

Key Words: sodium ▪ ion channels ▪ antiarrhythmia agents ▪ pharmacology ▪ electrophysiology ▪ genes

The congenital long-QT syndrome is an inherited cardiac disorder defined by prolonged ventricular repolarization, recurrent syncope, a propensity to polymorphous ventricular tachycardia (torsades de pointes), and sudden death.1,2 Molecular genetic studies have identified defects in the α-subunit C-terminus of the Na+ channel α-subunit, are linked to 1 form of the disease: LQT-3.3 Initial functional analysis of most SCN5A mutations has revealed mutant Na+ channels that fail to inactivate completely on prolonged depolarization,4–8 a property sufficient to delay repolarization of the ventricular action potential and increase vulnerability of the heart to arrhythmias.9 In contrast, the D1790G (DG) SCN5A mutation,10 located in the cytoplasmic region of the α-subunit C-terminus, causes a marked negative shift in the relationship between channel availability and membrane potential and alters inactivation kinetics of mutant channels11 but does not promote sustained inward current.7

Pharmacological analysis of LQT-3 mutant channels expressed heterologously has provided evidence that sodium channel blockers that interact with either the inactivated or open state of the channel12,13 effectively block maintained current conducted by mutant channels,14–20 shorten action potential duration in cellular studies,21,22 and in preliminary studies correct QT prolongation in patients.22,23

Because the biophysical properties of the DG mutation do not promote maintained current during the action potential plateau phase, it had been suggested that agents such as lidocaine would not be effective in correcting the disease phenotype linked to this mutation.7 Clinical studies24 have confirmed this prediction but, in addition, have shown that flecainide, which preferentially blocks open but not inactivated channels,25 is effective in correcting DG-induced QT prolongation in patients carrying the DG gene defect. However, the mechanism underlying this mutation-specific therapeutic efficacy has not yet been determined.

Here, we report the pharmacological profile of DG channels expressed in a mammalian cell line and show that this point mutation confers a flecainide sensitivity that is distinct from wild type (WT) and at least 1 other LQT-3 mutant channel.20 Our results reveal marked drug-specific differences in channel modulation that are consistent with the clinical efficacy of both lidocaine and flecainide and suggest that over concentration ranges that are used clinically, it is the marked difference in flecainide’s use-dependent block (UDB)
of DG compared with WT channels that underlies its therapeutic efficacy. The results of this study provide further support for the approach of mutation-specific pharmacology as a basis for the management of inherited cardiac arrhythmias.

Methods

Expression of Recombinant Na⁺ Channels

HEK 293 cells (Cold Spring Harbor Laboratories) were grown under culture conditions and transfected with equal amounts of Na⁺ channel α- (WT or DG, respectively), hβ₂, and/or hβ₄-subunit cDNAs subcloned individually into the pcDNA3 (Invitrogen) vector (total cDNA, 2.5 μg) by a lipofection procedure previously described by us. Control experiments (data not shown) indicated no significant differences in channel activity with or without drug for these subunit combinations. β-Subunit cDNAs were gifts of Drs L. Isom (University of Michigan, hβ₄) and A. George (Vanderbilt University, hβ₂), and the DG mutation was constructed as previously described.

Electrophysiology

Membrane currents were measured by whole-cell patch-clamp procedures with Axopatch 200B amplifiers (Axon Instruments) and the following solutions (mmol/L): internal: CsCl 60, aspartic acid 50, CsOH. Drug (Sigma Chemical Co) solutions were made from stock solutions in 10 mmol/L (flecainide) or 100 mmol/L (lidocaine) stock solutions in H2O. Experiments were carried out with pClamp7 software (Axon Instruments), and data were analyzed with Origin software (Microcal Software). Unless otherwise specified in the figure legends, experiments were carried out at room temperature (22°C). Measurements at higher temperature were performed with a solution heater (In-line Heater SH-27B, Warner Instrument Corp) warming the superfusate to 37°C. Recordings were made during 25-ms test pulses to −10 mV from −100-mV holding potentials. Tonic block (TB) was measured at 0.033 Hz after steady state was achieved in the presence of drug (1 minute for lidocaine and 2 to 4 minutes for flecainide). Steady-state inactivation was measured with 5-second conditioning pulses followed by a test pulse (−10 mV), with an interpulse interval of 30 seconds. Steady-state UDB was reached in response to trains of variable numbers of pulses (100 to 600, −10 mV) at frequencies indicated in the figure legends. UDB was measured as block induced by pulse trains relative to TB for a given drug concentration. UDB data were normalized to currents recorded with the same protocols but in the absence of drug.

Data are represented as mean±SEM. Two-tailed Student’s t test was used to compare means; a value of P<0.05 was considered statistically significant.

Results

TB of WT and Mutant Channels

Because the DG mutation alters the voltage-dependence of steady-state inactivation of expressed channels, we first tested for differences between the interactions of lidocaine and flecainide with the inactivated state of WT and DG channels. Figure 1 shows that lidocaine, but not flecainide, induces marked hyperpolarizing shifts in the steady-state inactivation relationship for both WT and DG channels. Furthermore, the effects of lidocaine on inactivation are approximately the same for WT and DG channels.

Figure 1. Effects of flecainide and lidocaine on steady-state inactivation of WT and DG channels. Steady-state availability of channels was measured (Methods) in absence and presence of each drug. A and B. Current traces, normalized to peak drug-free currents, illustrate effects of lidocaine (300 μmol/L) and flecainide (30 μmol/L). Arrows indicate drug-induced changes in peak currents for each condition. C and D. Averaged steady-state availability curves are shown in absence (circles) and presence of lidocaine (300 μmol/L, squares) and flecainide (30 μmol/L, triangles) for WT (C) and DG (D) channels. Na⁺ currents elicited by −10-mV test pulses were normalized to largest currents obtained in control conditions from hyperpolarized holding potentials. Graphs show normalized current plotted against conditioning pulse voltage. Smooth lines are according to 1/(1+exp[(Vc−V½)/k]), where Vc is conditioning potential, V½ is voltage for which half the channels are not available, and k is a slope factor. V½ (mV) for WT is −66.2±0.9 for control, −69.8±1.4 with flecainide, and −85.7±1.4 with lidocaine; V½ (mV) for DG is −81.8±1.4 for control, −87.3±2.4 with flecainide, and −102.5±2.7 with lidocaine; n=4 cells per condition.

2. However, at clinically relevant concentrations of lidocaine (30 μmol/L) and flecainide (3 μmol/L), neither drug discriminates between WT and DG channels on the basis of TB.

UDB: Distinctions Between WT and DG Channels

Therefore, we next compared block that accumulates with repetitive activity when DG mutant and WT channels are exposed to lidocaine and flecainide (Figure 3). Again at clinically relevant concentrations (30 μmol/L), there is no difference between lidocaine block of WT and DG channels (Figure 3A). In contrast, there is a statistically significant (P<0.001) difference between flecainide UDB of DG and WT channels at the clinically relevant concentration of 3 μmol/L. This difference is evident over a broad concentration range: EC₅₀ for flecainide UDB of DG channels is roughly 5 times lower than for block of WT channels (Figure 3B). In contrast, UDBs of WT or DG channels by lidocaine are approximately the same over all concentrations tested. In addition, the distinction in use-dependent drug action between WT and DG channels is retained when the frequency range of pulse application is extended to a broader frequency range (Figure 3C and 3D).

Effect of DG Mutation on Recovery From Flecainide Block

Block that accumulates as a consequence of repetitive channel activity (UDB) is caused by a balance between the time...
course of the onset of block (during depolarization) and the recovery from block (during repolarization). To understand the marked sensitivity of DG channels to UDB, we next investigated the time course of the recovery from UDB. Here, we focused only on the effects of flecainide, because there was little difference between WT and DG channels in response to UDB by lidocaine (Figure 3A and 3C).

In these experiments, we applied a "conditioning" train of pulses for a fixed duration and frequency to induce flecainide block of channels. As illustrated in Figure 4, in the absence of drug, DG channels tend to recover faster from inactivation that occurs as a consequence of the conditioning train. In the presence of flecainide, repriming of channels is very different: now DG channels recover very slowly. Even after 10 seconds at the holding potential (−100 mV) under pulse-free conditions, only a small fraction of the flecainide-blocked current recovers. This result suggests that the flecainide-bound DG channel is very stable and that infrequent pulsing can still be very effective at accumulating block because once blocked, channels remain nonconducting for tens of seconds.

**Clinical Efficacy of Flecainide: Distinctions Between UDB of WT and DG Channels**

The dramatic slowing of flecainide unblock caused by the DG mutation (Figure 4) has important implications for the clinical usefulness of this compound in the treatment of LQT-3 in...
carriers of the DG mutation. Because use-dependent flecainide block discriminates between WT and DG mutant channels (Figure 3), it is important to demonstrate UDB of DG channels under conditions that more closely resemble those encountered in the heart. Thus, we tested for differences between WT and DG channels in the response to flecainide, when longer pulses (400 ms), which mimic the duration of action potentials in LQT-3 patients, are applied at a physiological frequency (1 Hz) and temperature (37°C). These experiments (Figure 5) confirm that, even under these conditions, the extremely slow recovery from flecainide block of DG channels is sufficient to cause significantly greater block of DG versus WT channels.

Discussion

Molecular Basis for Mutation-Specific Pharmacology: Implications for a Role of C-Terminus Inactivation and Drug Activity

The principal finding of this study is that a LQT-3–linked mutation of the heart α-subunit of the Na⁺ channel dramatically and specifically changes the manner by which channels encoded by the mutant gene interact with sodium channel–blocking drugs. The mutation confers a higher sensitivity to UDB by flecainide, but not lidocaine, over a clinically relevant concentration range. Flecainide TB is not affected by the DG mutation. Our findings thus illustrate the importance of investigating mutation-induced changes not only in channel function but also in channel pharmacology.

Voltage-dependent block of Na⁺ channel currents by antiarrhythmic drugs is a consequence of distinct interactions with different states of the voltage-gated Na⁺ channel. Lidocaine and flecainide differ in their modes of action in that lidocaine interacts preferentially with inactivated channels, and drug block is not necessarily dependent on channel openings, whereas flecainide requires channels to open and is not dependent on channels entering the inactivated state to promote block.

We observed that the DG mutation does not influence the interaction of lidocaine with the Na⁺ channel, even if TB was greater than for mutant channels (Figure 2). Indeed, this effect is explained by the fact that lidocaine shifts the steady-state inactivation curve by the same amount for WT as for the DG mutant channel, but the DG mutation by itself already shifts this curve by ~20 mV in the absence of drug. Because flecainide has a much weaker effect on the inactivation curve (Figure 1), there is little difference between flecainide-induced TB of WT and DG channels. In contrast, the DG mutation markedly increases flecainide UDB of channels, in large part because of the pronounced slowing of the repriming of DG channels in the presence of the drug (Figure 4).

This specific alteration of the sensitivity to UDB by flecainide is not a general property of all LQT-3 mutant channels. Recently, Nagatomo et al found that LQT-3 ΔKPQ mutant channels have an intrinsically higher affinity than WT channels to flecainide, but in the case of this mutation, sensitivity to both TB and UDB is increased. Thus, our data indicate that the DG point mutation causes a unique pharmacological response of the expressed channels, which is distinct not only from WT but also from ΔKPQ mutant channels.

The DG mutation is a nonconservative change from an aspartic acid to a glycine only 18 amino acids away from transmembrane segment S6 in the C-terminus of the Na⁺ channel α-subunit, a region of the channel not previously considered to play a major role in the molecular interactions of flecainide. Our data clearly show that this is not the case and raise the possibility that other residues of the C-terminus may also be important in determining pharmacological responses of Na⁺ channels. Importantly, 1 other nonconservative C-terminus LQT-3 mutation (E1784K) has been reported recently, and a C-terminus insertion mutation (1795insD) has been linked to both Brugada’s syndrome and LQT-3. Our work strongly suggests that these mutations may also modify the interactions of the encoded channels by flecainide (and probably other drugs), raising the possibility of pharmacological targeting of a broad range of mutation-induced phenotypes. As has been shown for ΔKPQ and DG channels, however, determination of the pharmacological profile must be carried out systematically on a mutation-by-mutation basis before this would be possible.

Relationship Between Molecular Pharmacology and Therapeutic Efficacy

Our data on recombinant human Na⁺ channels complement those of Benhorin et al., which have shown that flecainide, but not lidocaine, significantly decreased the QTc interval in DG carriers by ~10% but was without effect in control patients. This difference was even more striking when the effect of flecainide on the marked repolarization heterogeneity seen in DG carriers was considered. Our experiments indicate that over the clinically relevant drug concentration range and under physiological conditions, flecainide discriminates between WT and DG primarily because of the pronounced effect of the DG mutation on flecainide UDB. The correlation between the clinical results and our data strongly suggests that it is this mechanism of action that underlies the therapeutic usefulness of flecainide compared with lidocaine in the treatment of carriers of the DG mutation. Interestingly,
flecainide has also recently been shown to be very effective in treating carriers of the ΔKPQ LQT-3 mutation (A.J. Moss, personal communication), even though, as discussed above, the interactions of flecainide with ΔKPQ and DG mutant channels differ. In the case of both channel defects, however, recovery from the drug-blocked state is markedly slowed compared with WT channels, and it may be this common mode of action that makes this drug so useful as a therapeutic tool in the treatment of carriers of these gene defects.

In summary, we have found that the LQT-3 DG mutation changes the pharmacological response of encoded channels in a manner that differs not only from WT but also from other LQT-3 mutant channels. The pharmacological profile of DG channels shows distinct changes that occur over a therapeutically relevant concentration range. Our data provide further support for the usefulness of a mutation-specific pharmacological approach for the management of distinct inherited ion channel defects.

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References

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