

Preferential Block of Late Sodium Current in the LQT3 Δ KPQ Mutant by the Class I_C Antiarrhythmic Flecainide

TOSHIHISA NAGATOMO,¹ CRAIG T. JANUARY, and JONATHAN C. MAKIELSKI

Department of Medicine, Section of Cardiology, University of Wisconsin, Madison, Wisconsin

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ABSTRACT

Flecainide block of Na⁺ current (I_{Na}) was investigated in wild-type (WT) or the long QT syndrome 3 (LQT3) sodium channel α subunit mutation with three amino acids deleted (Δ KPQ) stably transfected into human embryonic kidney 293 cells using whole-cell, patch-clamp recordings. Flecainide (1–300 mM) caused tonic and use-dependent block (UDB) of I_{Na} in a concentration-dependent manner. Compared with WT, Δ KPQ I_{Na} was more sensitive to flecainide, and flecainide preferentially inhibited late I_{Na} (mean current between 20 and 23.5 ms after depolarization) compared with peak I_{Na}. The IC₅₀ value of peak and late I_{Na} for WT was 127 \pm 6 and 44 \pm 2 μ M (n = 20) and for Δ KPQ was 80 \pm 9 and 19 \pm 2 μ M (n = 31) respectively. UDB

of peak I_{Na} was greater and developed more slowly during pulse trains for Δ KPQ than for WT. The IC₅₀ value for UDB of peak I_{Na} for WT was 29 \pm 4 μ M (n = 20) and for Δ KPQ was 11 \pm 1 μ M (n = 26). For Δ KPQ, UDB of late I_{Na} was greater than for peak I_{Na}. Recovery from block was slower for Δ KPQ than for WT. We conclude that Δ KPQ interacts differently with flecainide than with WT, leading to increased block and slowed recovery, especially for late I_{Na}. These data provide insights into mechanisms for flecainide block and provide a rationale at the cellular and molecular level that open channel block may be a useful pharmacological property for treatment of LQT3.

The congenital long QT syndrome (LQT) is an inherited cardiac disorder that causes ventricular arrhythmias, resulting in syncope and sudden death. One form of the disease, LQT3, is caused by mutations in *SCN5A*, the gene that encodes the voltage-dependent cardiac Na⁺ channel α subunit in humans (hH1) (Jiang, 1994; George, 1995; Wang et al., 1995a,b). A KPQ deletion (Δ KPQ: lysine, proline, and glutamine at positions 1505–1507) in the linker between domains III and IV of hH1 is the most common mutation associated with LQT3. The Δ KPQ mutant channel exhibits late channel openings caused by a defect in inactivation (Bennett et al., 1995; An et al., 1996; Dumaine et al., 1996; Wang et al., 1996c), and the resulting late Na⁺ current (I_{Na}) would prolong the action potential and cause QT prolongation on the surface ECG.

Gene-specific therapy for LQT3 (i.e., the use of drugs that target the Na⁺ channel, more specifically, late I_{Na}) is a logical approach. Most antiarrhythmic drugs block the Na⁺

channel in a use-dependent manner by preferential binding to either the inactivated state or the open state as described in the modulated receptor model (Hille, 1977; Hondeghem and Katzung, 1977). Inactivated state blockers of the Class Ib antiarrhythmic grouping (e.g., mexiletine) inhibit late I_{Na} at the cellular level (Wang et al., 1997), shorten action potential duration in a cellular model of LQT3 (Priori et al., 1996; Shimizu and Antzelevitch, 1997), and shorten the QT interval in LQT3 patients (Schwartz et al., 1995). We hypothesized that an open channel blocker would also be effective (perhaps more so) because of the prolonged dwell time of LQT3 channels in the open state. To test this hypothesis, we studied the Class Ic antiarrhythmic drug flecainide, a predominant open state blocker, comparing tonic block and use-dependent block (UDB) by flecainide for peak and late I_{Na} for the wild-type (WT) human cardiac Na⁺ channel and the Δ KPQ mutant. Our findings may provide a molecular mechanism for the recently observed correction of the QTc interval in the electrocardiograms of Δ KPQ LQT3 patients by flecainide (Windle et al., 1999).

Materials and Methods

Clones and Construction of Δ KPQ Mutation. The human heart Na⁺ channel clone we used (hH1a) was kindly provided by Dr. H. Hartmann (Baylor College of Medicine, Houston, TX). The nucle-

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¹ Current affiliation: Second Department of Internal Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan.

ABBREVIATIONS: LQT, long QT syndrome; Δ KPQ, LQT3 sodium channel α subunit mutation with three amino acids deleted; I_{Na}, Na⁺ current; UDB, use-dependent block; WT, wild-type; MEM, minimal essential medium.

Results

Tonic Block for Peak and Late I_{Na} is Greater for Δ KPQ.

Tonic block is defined as the decrease in I_{Na} in the presence of drug and in the absence of previous depolarizations or with a frequency of depolarizations sufficiently slow that no UDB developed. I_{Na} in response to a 24-ms depolarization to -20 mV from a holding potential of -150 mV was measured in control solutions and 5 to 7 min after exposure to various concentrations of flecainide (see example records in Fig. 1, A and B). Tonic block of peak I_{Na} was assessed by dividing the peak I_{Na} for the first depolarization in flecainide by the control peak I_{Na} . The concentration dependence of block (Fig. 1, C and D) was then fit with a single site-binding equation. The half concentration (IC_{50}) for block of peak I_{Na} was $127 \pm 5.6 \mu\text{M}$ for WT ($n = 20$) and $80 \pm 9.0 \mu\text{M}$ for Δ KPQ ($n = 31$), a significant difference ($P < .001$). To assess the tonic block of late current, the average value of I_{Na} between 20 ms and 23.5 ms after the depolarization was measured in control and flecainide (see Fig. 1, A and B, for examples) and analyzed in the same way as for peak I_{Na} (Fig. 1, C and D). The IC_{50} value for block of late I_{Na} was $44 \pm 1.6 \mu\text{M}$ for WT ($n = 20$) and $19 \pm 1.6 \mu\text{M}$ for Δ KPQ ($n = 31$), a significant difference ($P < .001$).

Use-Dependent Block of Peak I_{Na} Is Greater for Δ KPQ.

Use-dependent block of peak I_{Na} was produced by imposing a pulse train of 200 depolarizations of 24-ms duration from -150 mV to -20 mV at 5 Hz. Under control conditions, application of this pulse train produced negligible ($<5\%$) change in peak I_{Na} (data not shown). Figure 2A shows an example of UDB by $100 \mu\text{M}$ flecainide for WT and Δ KPQ. Figure 2B shows summary data for the relative amplitude of peak I_{Na} (compared with peak I_{Na} in the first pulse) in various concentrations of flecainide plotted versus the pulse number in the train. UDB of peak I_{Na} was deeper but developed more slowly for Δ KPQ than for WT. The time course of UDB was evaluated by fitting the peak currents to a one- or two-component exponential decay as shown in Fig. 2B with summary data in Table 1.

The steady-state UDB was evaluated as the decrease in peak I_{Na} for the 200th depolarizing pulse compared with the first depolarizing pulse. Dose-response curves for the UDB of peak I_{Na} (Fig. 3) were fit to a single site-binding equation and yielded an IC_{50} value of $29 \pm 4.4 \mu\text{M}$ for WT ($n = 20$) and $11 \pm 1.0 \mu\text{M}$ for Δ KPQ ($n = 26$), again showing that Δ KPQ is significantly ($P < .001$) more sensitive to UDB by flecainide than WT. The steady-state UDB of the late currents were also analyzed. For WT UDB, the late currents were too small to be measured reliably (Fig. 3); for Δ KPQ, they could only be measured for the lower doses (Δ , $n = 18$). The results show that the late current amplitude was preferentially blocked. Note that UDB for the late current does not follow a single-site-binding curve, nor, on closer inspection, does UDB for peak current. The amplitude of UDB has a complex dependence on uptake and recovery from block during repetitive depolarization and repolarization (e.g., Starmer, 1987). Thus the fits to the peak current should be regarded as descriptive and not mechanistic.

Recovery from Use-Dependent Block of Peak I_{Na} Is Slower for Δ KPQ. We measured recovery rates after UDB induced by a train of 100 24-ms depolarizing steps to -20 mV at 25 Hz in $10 \mu\text{M}$ flecainide. The pulse train was followed by a variable recovery interval (ΔT) and a test depolarization (Fig. 4, inset). Summary results for normalized peak I_{Na} in

otide and amino acid numbering follow Hartmann et al. (1994). The Δ KPQ mutation was kindly provided by Drs. John W. Kyle and Gayle S. Tonkovich (University of Chicago, Chicago, IL). It was made by polymerase chain reaction techniques as described previously (Nagamoto et al., 1998). The entire polymerase chain reaction-generated region was completely sequenced to confirm the deletion and ensure that no unwanted changes were made in the channel.

Cell Preparation and Transfection. Cells from transformed human embryonic kidney cell line 293 were used. Approximately 5×10^5 cells were seeded on a 60-mm diameter plate (Falcon 3001) with 3 ml of culture medium 1 day before the transfection. Culture medium was MEM complete medium containing: minimum essential medium (Eagle's salts and L-glutamine), 10% fetal bovine serum, 2 mM L-glutamine, 0.1 mM MEM nonessential amino acids solution, 1 mM MEM pyruvate solution, 10,000 U of penicillin and 10,000 μg of streptomycin. Transfection was carried out using a cationic liposome method. Details have been described previously (Nagamoto et al., 1998).

Electrophysiological Recordings. I_{Na} was recorded using the whole-cell patch clamp method. The bath solution contained 140 mM NaCl, 4 mM KCl, 1.8 mM CaCl_2 , 0.75 mM MgCl_2 , and 5 mM HEPES, pH 7.4, set with NaOH. The pipette solution contained 120 mM CsF, 20 mM CsCl_2 , 5 mM EGTA, and 5 mM HEPES, pH 7.4, set with CsOH. Electrodes were made from borosilicate glass with a puller (P-87; Sutter Instrument, Novato, CA) and heat-polished with a microforge (MF-83; Narishige, Tokyo, Japan). They had a final resistance of less than 1.2 M Ω in the whole-cell recording when filled with the pipette solution. Cells for study were placed in a Plexiglas chamber with continuously flowing bath solution mounted on an inverted microscope (Nikon, Tokyo, Japan) in a Faraday cage. Membrane currents were recorded with an Axopatch 200 amplifier (Axon Instruments Inc., Burlingame, CA) and data were acquired using pClamp v6.03. Data were digitized at 100 kHz and low pass filtered at 10 kHz.

Adequate voltage control for whole-cell currents was achieved using low resistance pipettes, highly conductive solutions, and series resistance compensation. To further minimize possible errors arising from loss of voltage control, we selected clonal lines expressing lower I_{Na} density. The small size and low capacitance (<10 pF) of these cells allowed for very rapid charging of the membrane capacitance. We required the presence of two indices of voltage control: a graded slope of the activation curve (Boltzmann slope factor > 5.0) and scaling of currents of different amplitudes to the same test potential as in steady-state inactivation and recovery protocols. These indirect measures have been validated in other preparations (Makielski et al., 1987). With the whole-cell, ruptured patch technique, intrinsic kinetic changes with time have been reported (Makielski et al., 1987; Wang et al., 1996a). A shift in steady-state inactivation of < 0.5 mV/min was observed in our preparation. To minimize the effects of this shift, flecainide was applied rapidly and block was measured within 5 to 7 min except for recovery protocols.

Data Analysis. Leak subtraction of peak and late currents was calculated by extrapolating holding currents at subthreshold potentials (≤ 100 mV) to the potential of interest (Makielski et al., 1987). Data were fit to model equations using nonlinear regression (pClamp v6.03 or SigmaPlot 3.0). Parameter estimates are reported \pm S.E. of the parameter estimate; statistical differences in parameters were determined by a t statistic. Goodness of fit was judged both visually and by the sum of squared errors. The number of exponential components that best fit the data were determined by an F -ratio test ($P < .05$) to account for the increased number of free parameters. Experiments at different concentrations of flecainide were done in separate cells at the same time after patch rupture. Summary data are expressed as means \pm S.E. n represents the number of cells studied. In figures, the symbols represent mean data and the error bars are shown only when they exceed the size of the symbol.

response to the test depolarization are plotted versus a logarithmic scale of the recovery interval (Fig. 4). For control conditions, two components of recovery are apparent, with >95% of the recovery occurring within 10 ms for both WT and Δ KPQ. This is consistent with rapid recovery from inactivation of unblocked channels (An et al., 1996; Wang et al., 1996c). The small amplitude of the slow recovery component in control (<5%) indicates that the relatively short (24 ms) conditioning depolarizations are not sufficiently long to induce slow recovery (Shander et al., 1995). In 10 μ M flecainide, three components of recovery are apparent (Fig. 4) for both WT and Δ KPQ. The fastest component (<10 ms) is consistent with recovery of unblocked channels from inactivation, an intermediate component (10 ms–1000 ms) is consistent with a rapid recovery from flecainide block, and the slowest component (>1000 ms) is consistent with a slow recovery from flecainide block. For recovery intervals <10 ms, recovery was faster for Δ KPQ compared with WT consistent with recovery from inactivation of unblocked channels. For recovery intervals >10 ms, the recovery rate from flecainide block was decreased for Δ KPQ compared with WT, especially for the first component of recovery. Results from recovery in the presence of 3 μ M flecainide were similar to those of 10 μ M (data not shown). This slower recovery for Δ KPQ is consistent with the deeper UDB found for the mutant (Fig. 2) and it is also consistent with the slower devel-

opment of block. Quasi-equilibrium for the amplitude of UDB is reached when the number of channels blocked during a pulse is equal to the number of channels being unblocked during the recovery interval. Slower rates of interaction during either interval will slow the process of achieving this quasi-equilibrium (Starmer, 1987).

Use-Dependent Block of Late I_{Na} Is Greater for Δ KPQ. Fig. 5 shows the time course of UDB of peak and late (averaged between 20 ms and 23.5 ms) I_{Na} for 3 mM flecainide. At this concentration, little tonic block was exhibited and it is near the levels achieved in patients treated for arrhythmia. For Δ KPQ, the late component of I_{Na} was more sensitive to UDB by flecainide than was peak I_{Na} as also shown in Fig. 3.

Flecainide Blocks the Open State of hH1. To confirm the affinity of flecainide for open state channels, we compared the amount of UDB for peak I_{Na} with various pulse durations in 100 μ M flecainide. If flecainide has important additional affinity for the inactivated state, then prolonging the depolarization would be expected to increase the level of block. Pulse trains of depolarizations to –20 mV with various pulse durations (1, 2, 5, 10, 20, 100, 200 ms) were applied from a holding potential of –150 mV and the recovery interval was kept constant at 200 ms. The fractional UDB in both WT and Δ KPQ saturated within the first 10 ms of the depolarization and less than 5% of block occurred after further pulse prolongation (Fig. 6). Although data such as these have

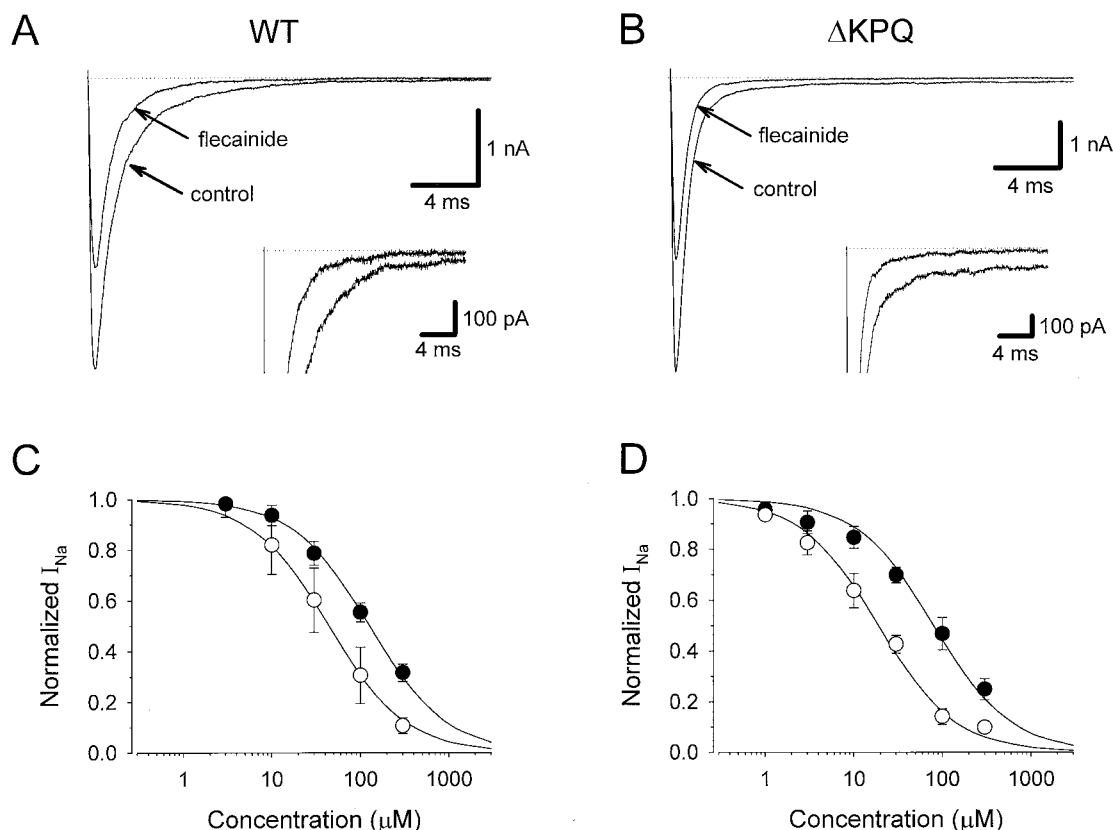


Fig. 1. Tonic block for peak and late I_{Na} . A and B, representative current recordings in control and in the presence of 100 μ M flecainide for WT and the Δ KPQ mutant hH1a. I_{Na} was elicited by a 24-ms step depolarization from –150 mV to –20 mV. Insets show magnified current traces for comparison of the late component. C and D, concentration dependence of flecainide-induced tonic block of I_{Na} . Current was measured at peak (●) and “late” (○; average value between 20 ms and 23.5 ms). The proportion of I_{Na} remaining in the presence of flecainide was normalized to I_{Na} in control solutions for different concentrations. Data points represent the mean for three to seven measurements; bars represent S.E. Solid lines represent fits to a single-site binding equation: $\text{Normalized } I_{Na} = 1/(1 + ([\text{flecainide}]/IC_{50}))$ where [flecainide] is the millimolar concentration and IC_{50} is the half-blocking concentration. The IC_{50} values for WT were 127 mM and 44 mM at peak and late, respectively, and for Δ KPQ, the IC_{50} values were 80 mM and 19 mM at peak and late, respectively.

traditionally been interpreted as indicating open state block (Anno and Hondeghem, 1990; Nitta et al., 1992), an alternative explanation that cannot be excluded includes preferential binding to another transiently available state, such as a preopen state.

Discussion

This study is the first description of the effects of a Vaughan-Williams Class Ic antiarrhythmic drug, the pre-

dominantly open channel blocker flecainide, on I_{Na} through human cardiac Na^+ channels and on I_{Na} through the ΔKPQ mutant of the LQT3 syndrome. Consistent with previous studies of flecainide block in nonhuman Na^+ channels (Anno and Hondeghem, 1990), our results show that flecainide blocked I_{Na} in both a tonic and use-dependent manner and that recovery from block was slow and had multiple components. For the first time, we have characterized block of the late component of I_{Na} , the current flowing after peak I_{Na} that

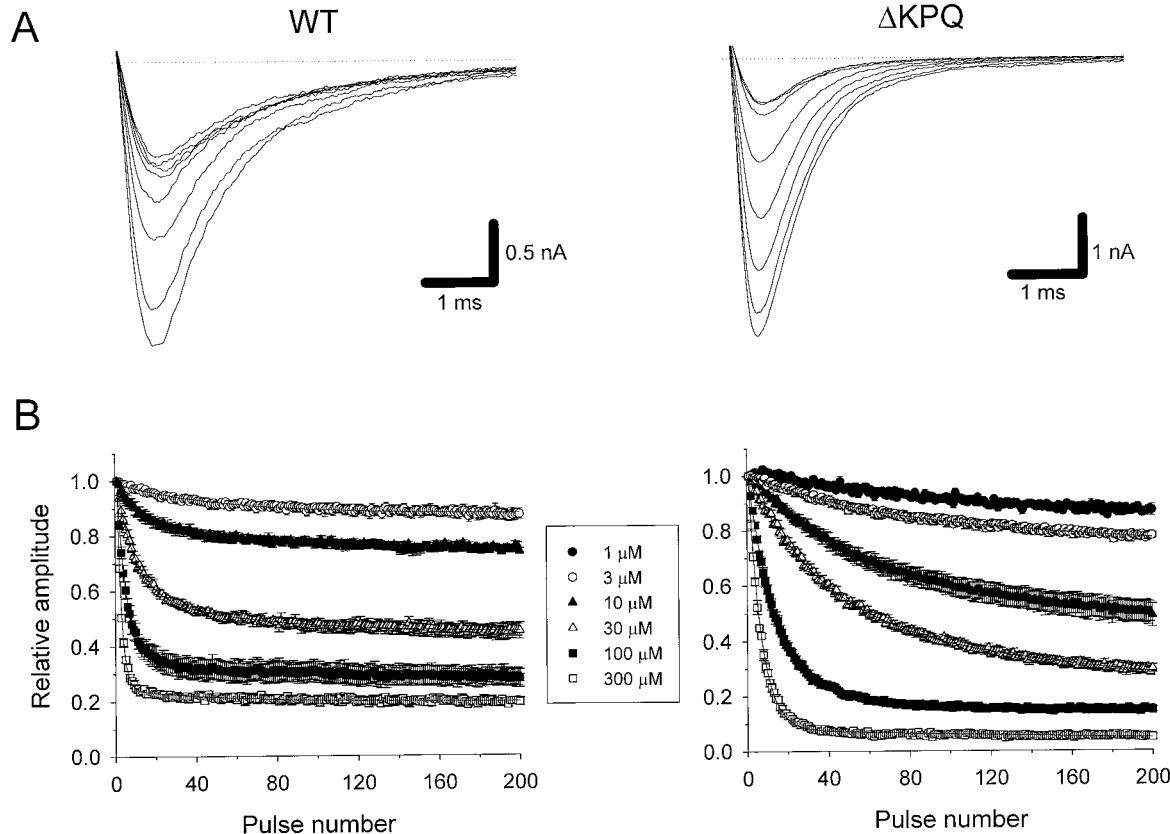


Fig. 2. Time course of use-dependent block of peak I_{Na} in response to pulse trains for WT and ΔKPQ hH1a channels. Pulse trains consisted of depolarizations of 24-ms duration from -150 mV to -20 mV applied at 5 Hz. A, superimposed current recordings in response to a pulse train in the presence of flecainide 100 mM. I_{Na} from the 1st, 2nd, 5th, 10th, 20th, 50th, 100th, and 200th pulse in the train are shown. B, time course of UDB for WT and ΔKPQ channels. Drug concentrations of 1 (●) (for ΔKPQ only), 3 (○), 10 (▲), 30 (△), 100 (■), and 300 μM (□) flecainide. Relative amplitude of I_{Na} for each pulse number was calculated as the peak I_{Na} for the pulse number divided by the peak I_{Na} for the first pulse in that train. Symbols represent the mean of three to five measurements; S.E. is shown as a bar. Solid lines represent fitting with a one or two exponential function with the parameters of the fit shown in Table 1.

TABLE 1
Parameters for Fitting UDB for 5-Hz Pulse Trains by Flecainide

	Concentration μM	n	τ_f (pulses $^{-1}$)	τ_s (pulses $^{-1}$)	A_s ($\times 10^{-2}$)	base ($\times 10^{-2}$)
WT	μM					
	3	4	54.5 ± 4.6			87.4 ± 1.9
	10	4	16.6 ± 6.8 ($n = 4$)	87.7 ± 20 ($n = 3$)	82.7 ± 1.6	74.0 ± 1.1
	30	4	8.5 ± 0.9	54.7 ± 7.7	60.4 ± 1.7	42.6 ± 2.7
	100	4	4.1 ± 0.4	69.0 ± 24	42.0 ± 3.6	27.9 ± 2.7
ΔKPQ	300	4	2.0 ± 0.11	49.5 ± 12	26.8 ± 1.7	19.5 ± 1.2
	1	3	121 ± 31			82.5 ± 3.5
	3	5	88.3 ± 14			$74.8 \pm 0.9^*$
	10	5	$66.7 \pm 4.7^*$			$47.8 \pm 4.0^*$
	30	5	$49.9 \pm 1.2^*$			$28.9 \pm 1.4^*$
	100	4	$10.6 \pm 0.5^*$	31.9 ± 1.3	$39.0 \pm 1.8^\dagger$	$14.9 \pm 0.8^*$
	300	4	$4.8 \pm 0.4^*$	$18.8 \pm 2.6^\dagger$	$26.8 \pm 7.3^\dagger$	$5.6 \pm 0.6^*$

The relative amplitude of peak I_{Na} was fit with a single-component ($A_f \times \exp(-t/\tau_f) + \text{base}$), or two-component ($A_f \times \exp(-t/\tau_f) + A_s \times \exp(-t/\tau_s) + \text{base}$) decay; t , pulse number; τ_f and τ_s , time constants of fast and slow components; A_f and A_s , fractional amplitudes of fast and slow components. Single component fits are reported where the F -ratio did not support fitting with two components (see *Materials and Methods*), and the τ 's for the single component fits were arbitrarily assigned to the τ_f column. n represents the number of experiments. Data = mean \pm S.E. Significant difference compared with WT at $P < .01$ (*) and $P < .05$ (†).

influences action potential duration especially for the Δ KPQ channel where this late component is increased. For Δ KPQ compared with WT, the open channel blocker flecainide 1) preferentially inhibited the late component of I_{Na} in both a tonic and use-dependent manner, 2) blocked peak and late I_{Na} with higher affinity, and 3) caused deeper UDB consistent with a demonstrated decrease in the recovery rate for flecainide for Δ KPQ current.

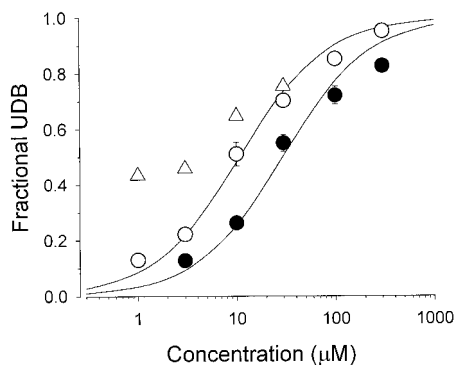


Fig. 3. Dose-response relationship for the UDB of peak and late I_{Na} by flecainide. Data points represent the mean relative decrease in amplitude of the peak current at the end of the pulse train for the results in Fig. 2 for WT (●) and Δ KPQ (○) peak I_{Na} . The solid lines were obtained by fitting data to a single site binding equation: Fractional UDB = [flecainide]/([flecainide] + IC_{50}) where [flecainide] is the micromolar concentration and IC_{50} is the half-blocking concentration. The IC_{50} values for WT (●) and Δ KPQ (○) peak I_{Na} were 29 μ M and 11 μ M, respectively. For Δ KPQ UDB, the late I_{Na} was measured as the relative decrease in amplitude for an average of the last 10 pulses in a 5 Hz train (see Fig. 5 at 3 mM) and mean data for 1, 3, 10, and 30 mM ($D, n = 18$) are shown. UDB of late I_{Na} at 100 and 300 μ M for Δ KPQ, and for all concentrations in WT, could not be measured because the current was too small.

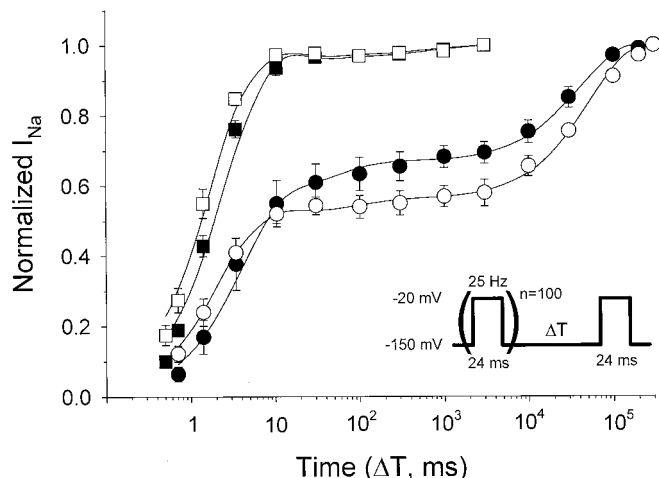


Fig. 4. Recovery from UDB of peak I_{Na} . UDB was induced by trains of 100 pulses at 25 Hz followed by a single test pulse after a variable recovery interval ΔT (see protocol inset). To compare the time courses of recovery, current amplitudes were normalized to the peak I_{Na} for the test pulse at 3 s in control and 300 s in the presence of 10 μ M flecainide. Symbols represent means; bars represent S.E. The averaged data were fitted with a two- (control) or a three- component (flecainide) exponential function: normalized $I_{Na} = 1 - [A_1 \times \exp(-t/\tau_1) + A_2 \times \exp(-t/\tau_2)]$ or $I_{Na} = 1 - [A_1 \times \exp(-t/\tau_1) + A_2 \times \exp(-t/\tau_2) + A_3 \times \exp(-t/\tau_3)]$, where t is the recovery time, τ_{1-3} are recovery time constants, and A_{1-3} are fractional amplitudes of each component. The fitted parameters were as follows: control (A_1, τ_1, A_2, τ_2): WT ($n = 8, \blacksquare$), 2.6 ms, 0.96, 909 ms, 0.04; Δ KPQ ($n = 10, \square$), 1.85 ms, 0.97, 833 ms, 0.03; and in flecainide ($A_1, \tau_1, A_2, \tau_2, A_3, \tau_3$): WT ($n = 5, \bullet$), 4 ms, 0.57, 55.6 ms, 0.1, 38.5 s, 0.33; Δ KPQ ($n = 10, \circ$), 2.2 ms, 0.52, 111 ms, 0.04, 52.6 s, 0.44.

The Δ KPQ Mutant Has an Intrinsically Increased Affinity for Flecainide. The modulated receptor (Hille, 1977; Hondeghem and Katzung, 1977) and the guarded receptor (Starmer, 1987) models provide two frameworks for understanding antiarrhythmic drug block in terms of intrinsic association and dissociation rates for ion channels. Our results show different affinities of flecainide for WT versus Δ KPQ channels and for peak versus late I_{Na} in Δ KPQ. Do

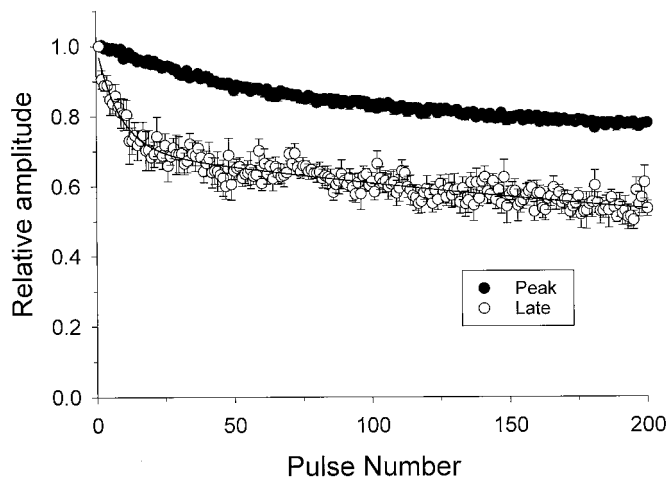


Fig. 5. Time course of UDB of late I_{Na} in response to pulse trains. UDB at peak I_{Na} (●) in the presence of 3 μ M flecainide was compared with the late I_{Na} (○), which was determined as the average current value between 20 ms and 23.5 ms. The data were normalized to the current amplitude of the first pulse in the train. The pulse train conditions are given in the legend to Fig. 2. Symbols represent means; bars represent S.E. ($n = 5$). The averaged data of the decrease in late I_{Na} was best fit with a double exponential function: Normalized $I_{Na} = A_f \times \exp(-t/\tau_f) + A_s \times \exp(-t/\tau_s) + \text{base}$, where t is the pulse number, τ_f and τ_s are time constants (in pulses) of fast and slow components, and A_f and A_s are fractional amplitudes of fast and slow components. The parameters of the fit were 0.30, 8.4, 0.39, 352, 0.31, for A_f, τ_f, A_s, τ_s , baseline, respectively.

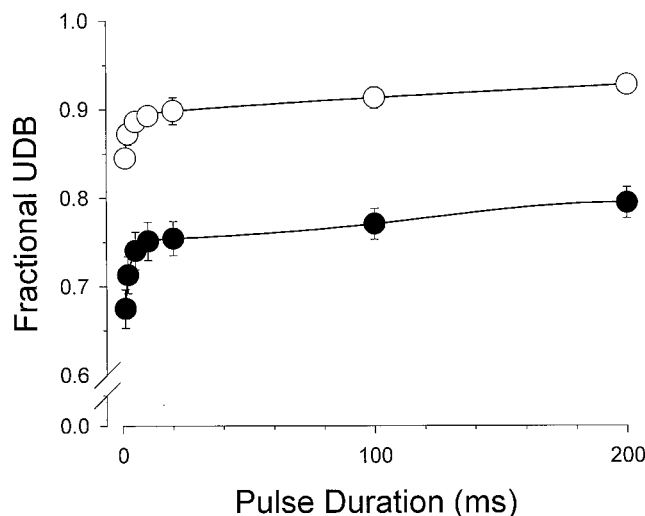


Fig. 6. Effect of pulse durations on UDB by 100 μ M flecainide. After 200 1-ms depolarizing pulses, trains of 50 depolarizing pulses with various durations (2, 5, 10, 20, 100, 200 ms) were applied with a constant recovery interval of 200 ms. Fractional UDB of peak I_{Na} for WT ($n = 5, \bullet$) and Δ KPQ ($n = 7, \circ$) are shown plotted for each of the pulse durations. The amount of fractional UDB was determined from the fitting of block development as described in Fig. 2. In a few cases (10-ms and 20-ms durations), the curve fits did not reflect the final level of block well and the average value of the steady-state normalized current amplitude in the last 10 pulses was used.

these results represent intrinsic affinity differences for drug binding to the channel protein, or are they instead secondary to the altered kinetics of Δ KPQ? Flecainide has been considered to be an open channel blocker (Anno and Hondeghem, 1990; Nitta et al., 1992), having higher affinity for the open state than the resting or inactivated states. Our data confirm for hH1 that flecainide has preferential affinity for a state transiently available at the beginning of the depolarization, consistent with open state block (Fig. 6). We originally hypothesized that flecainide might have a greater blocking effect on Δ KPQ because of an increased dwell time in the open state, rather than an intrinsic change in drug binding affinity. The results shown in Figure 6, however, suggest that use-dependent flecainide block occurred predominantly within the first 10 ms of depolarization for both WT and Δ KPQ channels. Therefore, additional binding to a persistent open state is unlikely to be an important mechanism for the higher affinity block in Δ KPQ channels. This suggests, instead, that the Δ KPQ channel has an intrinsically higher open state affinity for flecainide than WT.

The interpretation that the Δ KPQ mutant has an intrinsically increased affinity for flecainide is supported by additional findings. UDB of peak I_{Na} by flecainide was deeper (Fig. 2) and recovery from UDB was slower (Fig. 4) for Δ KPQ. Anno et al. (Anno and Hondeghem, 1990) interpreted flecainide recovery in terms of the modulated receptor hypothesis by suggesting that unbinding occurred rapidly via the open state and more slowly via the resting and inactivated states. In the present study, the Δ KPQ channel showed a dramatically reduced first component of recovery from flecainide block (between 10 ms and 3 s) compared with WT. Following the interpretation of Anno et al. (Anno and Hondeghem, 1990), our results show that flecainide unbound more slowly from the open state for the Δ KPQ channel, supporting the hypothesis that flecainide has an intrinsically higher affinity for the open state.

Tonic block is also greater for the Δ KPQ channel. Tonic block is usually interpreted as being caused by drug binding to the resting state; our data therefore support increased affinity of flecainide for the resting state of Δ KPQ. Alternatively, rapid drug binding to a preopen state, or to the open state before peak current is reached (Starmer et al., 1991), could also account for tonic block. Thus, the demonstrated increased affinity of flecainide for preopen or open states could account for both the increased tonic and UDB for the Δ KPQ channel. Our data do not distinguish between these possibilities.

Implications for the Antiarrhythmic Drug Binding Site. These data also provide additional evidence that the III-IV linker of the Na^+ channel participates as part of the binding site for antiarrhythmic drugs in addition to the previously implicated Domain IV S6 (Ragsdale et al., 1996). The binding site for flecainide is thought to be the same, or to at least overlap, the binding site for other antiarrhythmics and local anesthetic drugs such as lidocaine (Ragsdale et al., 1996). Modification of three key amino acids (IMF to QQQ) on the III-IV linker of the channel produced a channel that inactivated slowly and had a reduced affinity for lidocaine (Bennett et al., 1995). A naturally occurring mutation (T1313M) in the III-IV linker of the skeletal muscle Na^+ channel also slowed inactivation and decreased lidocaine affinity and UDB (Fan et al., 1996). For T1313M, the decreased

block was shown to be caused by an intrinsic change in channel affinity for the drug rather than secondary to the changes in inactivation properties, implicating the III-IV linker as a part of the binding site. Our results with flecainide show an increased intrinsic (that is, not explained by the altered channel kinetics) affinity difference for Δ KPQ over WT, supporting the view that the III-IV linker forms part of the binding site for the drug.

Clinical Implications and Limitations of the Study. Mexiletine, generally considered to be an inactivated state blocker, has attracted the most attention as a therapeutic agent for LQT3 patients (Schwartz et al., 1995; Shimizu and Antzelevitch, 1997; Wang et al., 1997). The present study provides direct evidence that supports open channel block as a pharmacological model for the treatment of LQT3 patients. Compared with antiarrhythmic drugs such as flecainide, Class Ib drugs, such as mexiletine, provide less UDB at slow heart rates because of more rapid recovery kinetics. These kinetic differences offer a theoretical advantage for flecainide as a gene-specific therapy in patients with LQT3. Flecainide has been beneficial in a skeletal muscle disease in which Na^+ channel mutants have increased late I_{Na} (Rosenfeld et al., 1997). Recently, it has been shown to correct the QTc interval in the electrocardiograms of Δ KPQ LQT3 patients (Windle et al., 1999). Caution must be used in extrapolating the IC_{50} values reported here to the clinical setting because of the experimental conditions required by the voltage clamp technique. These include the hyperpolarized holding potential (-150 mV) used to minimize the effects of time-dependent kinetic shifts (Makielski et al., 1987; Wang et al., 1996a) and the lower temperature (Johns et al., 1989; Makielski and Falleroni, 1991) used to facilitate voltage control. Two additional considerations may also affect the clinical application of these results. First, in the CAST study, flecainide was associated with increased mortality in patients after myocardial infarction (CAST investigators, 1989). Patients with LQT, however, generally do not have structural heart diseases and thus flecainide might be used safely. Second, flecainide is not entirely specific for I_{Na} . At relatively high concentrations, flecainide suppressed other currents, such as ATP-sensitive potassium current (Wang et al., 1995b), rapidly activating delayed-rectifier potassium current (Follmer et al., 1992; Wang et al., 1996b), the transient outward current (Wang et al., 1995b), and L-type calcium current (Scamps et al., 1989). Nonetheless, the efficacy of flecainide to shorten the QT interval in patients (Windle et al., 1999) suggests that late I_{Na} block is a predominant mechanism contributing to the shortening.

In conclusion, this study provides new insight into mechanisms of drug block of the human heart Na^+ channel and the Δ KPQ mutant channel of LQT3. Flecainide has higher intrinsic binding affinity for the mutant channel and a preference for blocking the late current. This may account for the correction of the QTc interval in the electrocardiograms of Δ KPQ LQT3 patients (Windle et al., 1999).

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Send reprint requests to: Jonathan C. Makielski, M.D., University of Wisconsin Clinics and Hospitals, 600 Highland Ave, H6/349, Madison, WI. E-mail: jcm@medicine.wisc.edu