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Selective Block of Late Currents in the ΔKPQ Na^+ Channel Mutant by Pilsicainide and Lidocaine with Distinct Mechanisms

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ABSTRACT

The congenital long QT syndrome is an inherited disorder characterized by a delay in cardiac repolarization, leading to lethal cardiac arrhythmias such as torsade de pointes. One form of this disease involves mutations in the voltage-dependent cardiac Na^+ channel, which includes an in-frame deletion of three amino acids (Lys-1505, Pro-1506, and Gln-1507; ΔKPQ). The potential for selective suppression of the mutant was examined by heterologous expression of ΔKPQ-Na^+ channels in Chinese hamster fibroblast cells via single-channel recording. In a single-channel cell-attached patch study, ΔKPQ-Na^+ channels yielded currents that peaked at ~1 ms after voltage steps to 0 mV with aberrant late currents, which were composed of burst and isolated openings. The affinity of certain anesthetics (pilsicainide and lidocaine) to the late currents of the mutant channels was examined. It was revealed that 1) pilsicainide (1 μM), an open channel blocker of voltage-dependent Na^+ channels, remarkably decreased the late currents primarily by the shortening of burst duration without suppressing the initial peak current; and 2) lidocaine (1 μM), an inactivated channel blocker, decreased the late currents primarily by the suppression of isolated channel openings. Because the late currents in ΔKPQ mutants are mainly composed of the burst openings, we conclude that pilsicainide is capable of selectively blocking the late currents in the mutant Na^+ channels that show dominant abnormal burst openings such as in ΔKPQ mutants.

The congenital long QT (LQT) syndrome is predominantly an autosomal-dominant disorder that is characterized by prolongation of the ventricular action potential and a propensity to ventricular tachycardia (torsade de pointes) and sudden death (Moss and Robinson, 1993; Schwartz et al., 1995a). One LQT locus on human chromosome 3 (LQT3) encodes the voltage-gated cardiac Na^+ channel α subunit (*hH1* and *SCN5A*) (Gellens et al., 1992; George et al., 1995). Several *SCN5A* mutations have been identified in DNA from affected members of LQT3 families (Wang et al., 1995; An et al., 1998; Makita et al., 1998), including the ΔKPQ mutant (deletion of residues Lys-1505, Pro-1506, and Gln-1507). A biophysical phenotype of ΔKPQ channels has been reported previously (Bennett et al., 1995a,b; Dumaine et al., 1996; Wang et al., 1996; Chandra et al., 1998), and inactivation defects have been clarified.

An important goal for treatment of this disease is to control the QT intervals by selective suppression of the phenotype produced by the mutation. Recent studies have demonstrated that late openings in ΔKPQ-mutant channels were suppressed by a high concentration of lidocaine (An et al., 1996) or by mexiletine (Dumaine et al., 1996) when expressed in *Xenopus laevis* oocytes. On the other hand, Wang et al. (1997) reported that relatively low doses of mexiletine inhibited the late currents (K_d = 2.1 μM) and the initial peak current (K_d = 6.5 μM) of the ΔKPQ channels expressed in a mammalian cell line, indicating a possible problem for studying drug effects in *Xenopus* oocytes because of their large lipophilic yolk. Because the channel block depends on the state of the channel and is influenced by the relative number of resting, open, and/or inactivated channels and the time course of interstate transitions (Hondeghem and Katzung, 1977; Starmer and Courtney, 1986) and because the ΔKPQ mutation alters channel inactivation, it is likely that interactions with local anesthetic antiarrhythmic agents for ΔKPQ channels differ depending on their blocking style, i.e., whether they are open channel blockers or inactivation blockers. Although previous studies have examined the actions of inactivation blockers (lidocaine, mexiletine), these drugs may not be optimal candidates for a therapeutic approach to the

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ABBREVIATIONS: ΔKPQ, deletion of residues Lys-1505, Pro-1506, and Gln-1507; LQT syndrome, long QT syndrome; WT, wild type; CHW, Chinese hamster fibroblast; I_{Na}, initial peak current of the Na^+ current; NPo, open probability of channel; $_b$NPo, open probability of channel derived from bursting openings; $_i$NPo, open probability of channel derived from isolated openings; τ_f, fast time constant; τ_s, slow time constant.

ΔKPQ channel, because their mechanism of action is to block inactivated rather than open channels (Bean et al., 1983; Bennett et al., 1995a). We reasoned that open channel blockers may opportunistically suppress the abnormal reopenings of ΔKPQ Na^+ channels. To test this hypothesis, ΔKPQ-mutant Na^+ channels were expressed in a mammalian cell line, and channel behaviors and inhibition by a local anesthetic open channel blocker and an inactivation channel blocker of Na^+ channels were investigated by the cell-attached macropatch-clamp method. We explored the actions of the open channel blocker pilsicainide, an orally available local anesthetic antiarrhythmic agent (class Ic type) (Inomata et al., 1989; Kodama et al., 1999), on the burst and isolated openings of ΔKPQ channels compared with those of lidocaine, a representative inactivation blocker. Our results show that late burst currents in ΔKPQ-mutant channels are much more sensitive to inhibition by pilsicainide than lidocaine. Unexpectedly, the late isolated openings are sensitively suppressed by lidocaine. Such a difference can be exploited to develop a new therapeutic approach to management of QT intervals that are related to several different phenotypic LQT3 forms of the disease, based on the molecular pharmacology of the Na^+ channels.

Materials and Methods

Expression of Wild-Type (WT) and ΔKPQ Human Cardiac Na^+ Channels. Site-directed mutagenesis of human heart Na^+ channels (Gellens et al., 1992) was performed to construct ΔKPQ-mutant channel cDNA as described (Bennett et al., 1995b; Wang et al., 1996). Mutant and WT cDNAs were subcloned into pRc CMV (Invitrongen Corp., San Diego, CA) for expression in mammalian cells (Wang et al., 1996). Multiple independent recombinants were sequenced thoroughly in the mutated region and tested for expression studies. Chinese hamster fibroblast (CHW 1102) cells were purchased from Coriell Cell Repositories (Camden, NJ) and maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 29.2 mg/l L-glutamine in an atmosphere of 95% O_2 plus 5% CO_2 at 37°C on fibronectin-coated plastic coverslips in 16-mm tissue culture wells. For transient expression of the channels in CHW 1102, we prepared the following DNA solutions: 15 μg of plasmid DNA-encoding channels (pRc CMV-hH1 3′-UT for WT *hH1* and pRc CMV-hH1ΔKPQ for ΔKPQ), 4 μg of plasmid-encoding cell surface antigen (pCD EBO-Leu-2), and 10 μg of salmon sperm DNA each in 0.5 ml of $CaCl_2$ (250 μM) and (2×) DNA precipitation buffer (50 mM HEPES, 1.5 mM Na_2HPO_4,10 mM KCl, 280 mM NaCl, 12 mM glucose, pH 7.05; 5Prime → 3Prime Inc., Boulder, CO). After a 20-min incubation at room temperature, the DNA solution was added to a cell culture (in a 50-ml flask) that was 30 to 50% confluent. After 6 h at 37°C, the transfected cells were replated onto 35-mm glass-bottomed culture dishes (which also served as recording chambers) containing 1.5 ml of fresh Dulbecco's modified Eagle's medium. One microliter of Dynabeads M-450 CD8 (1.4×10^8 beads/ml; Dynal, Oslo, Norway) was added to the culture dishes before recording to discriminate cells expressing CD8.

Electrophysiological Recordings and Data Analysis. For electrophysiological measurements, cells were seeded onto cover glasses and incubated for 1 to 3 days in culture medium with fetal calf serum. Patch-clamp current recordings (List EPC-7; Darmstadt, Germany) were made in the cell-attached configuration (Hamill et al., 1981). Patch pipettes with resistances ranging between 0.4 and 1.0 MΩ were used to record currents from patches with 10 to 30 channels (macropatch). In step-pulse protocols, patches were depolarized for 95 ms or 195 ms every 2 s from a holding potential of −120 mV. Experimental protocols and data acquisition were performed with a DOS-based 486 microcomputer programmed with ASYST 3.0 (Asyst Software Technologies, Rochester, NY). Channel currents were eight-pole Bessel filtered at 2 kHz and digitized at 12 bits at 10 kHz or filtered at 3.5 kHz and digitized at 20 kHz. Data were analyzed with custom programs written in ASYST. Data were leak and capacity corrected with the scaled average of sweeps below threshold, i.e., without activity. An opening was identified by the presence of two successive data points above the 50% amplitude of the single-unit opening. Amplitude histograms were constructed from corrected traces with a bin width of 48.4 fA, and single-channel current was measured as the mean value from a Gaussian function fit to the amplitude histogram. For macropatches, current responses to depolarization to voltages below threshold were averaged and scaled to correct the data for leak and capacity from more positive potentials. Measurements of the peak currents were made from individual sweeps or averages of two to five sweeps. An estimate of the numbers of channels (N_o) in each macropatch was made from the following equation:

$$N_o = \frac{I_{Na}}{UA_p \cdot P_{open}},$$

where I_{Na} represents the peak I_{Na} at 0 mV. Based on measurements in patches containing a single channel, UA_p was set to the unitary channel amplitude at 0 mV (1.60 pA) and P_{open} to the open channel probability at 0 mV (0.49), when P_{open} was measured in separate experiments with a single-channel patch. This N_o was used for the calculation of open probability. NPo for late openings was calculated in individual sweeps by the proportion of time that the channels were open during each 185-ms duration, beginning 10 ms after the step to the end of the depolarization (195 ms) of 0 mV. Channel kinetics during the bursts were analyzed for the same late current period of 185 ms. The first and last events of each sweep were excluded from the analysis. Open and closed durations were place in various bins to check for effects of bin size; data are shown in 0.2-ms bins. Open and closed distributions were analyzed after discarding the first bin and were fitted with exponential functions. The bursting period was the time assigned from the onset of the depolarization to the onset of the first closed time that lasted >20 ms. The histograms were constructed and analyzed with a single-exponential curve fitting after discarding events that were not larger than 20 ms, because overlapping openings were occasionally observed for the initial 20 ms after depolarization. Ensembles were constructed from the average of sweeps at 0 mV. All experiments were performed at room temperature (20–23°C).

Solutions. The bath for the current recording contained 140 mM potassium aspartate and 10 mM HEPES (pH adjusted to 7.4 with CsOH). This solution was assumed to collapse the membrane potential so that the applied potential was considered to be the patch membrane potential. The pipette solution contained 280 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM tetraethylammonium (TEA) chloride, 10 mM HEPES, and 0.3 mM 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid with or without anesthetic agents (pH adjusted to 7.4 with TEA-OH). For some experiments, the pipette solution with anesthetic agent was filled only at the back side of the pipette to observe a delayed effect of the drug applied to the patched membrane. Electrode filling was performed as follows: The electrode tip was filled by simply dipping it into a small beaker containing the filling solution, which lacked anesthetic drug. With the tip geometry used for these studies, a brief dip of <1 s resulted in the movement of filling solution some 500 μm up into the tip. For most our work, we were able to obtain a GΩ seal to our cells within 2 min, so that we could observe Na^+ channel currents for 1 or 2 min before the anesthetic drug diffused to the tip and interfered with the channel, judged from the change in the initial peak currents (see *Results*). TEA^+ and 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid were added to block any existing endogenous K^+ and Cl^- channels. Pil-

sicainide (Suntory BioPharma Technology, Tokyo, Japan) and lidocaine were added to separate pipette solutions (from 10 mM stock solutions dissolved in distilled water). All chemicals except pilsicainide were purchased from Sigma Chemical Co. (St. Louis, MO).

Statistics. Data are summarized as means ± S.D. Responses of the initial peak current of the Na^+ current (I_{Na}) and the open probability (NPo) for pilsicainide or lidocaine were compared by the Mann-Whitney *U* rank-sum test. Whenever significance is indicated, Student's *t* test was used to determine significant difference between the pilsicainide and lidocaine groups, and the Bonferroni *t* test was used to determine significant difference between untreated and anesthetic groups. $P < .05$ was considered to be significant.

Results

Transfection of CHW cells with cDNA encoding the α-subunit of the LQT3-derived (ΔKPQ) cardiac Na^+ channel produced a 5 to 10% expression rate of functional voltage-dependent Na^+ channels in this study. In untransfected cells, we observed no actual inward current. Figure 1A shows a macropatch ΔKPQ-Na^+ channel activity of a transfected CHW cell. The activities were measured in the cell-attached mode during repetitive voltage-clamp pulses from a holding potential of −120 mV to the 0-mV test potential, at a rate of 0.5 Hz. The test potential of 0 mV was selected because it closely matches the plateau potentials of action potentials, in which the activated currents play a significant role in the action potential duration or the QT intervals in the ECG. Using relatively large electrode tips, we were able to record from patches that contained 10 to 30 Na^+ channels, i.e., a so-called macropatch. Macropatch channel recordings have the advantage that the initial peak current and the late single-channel activities can be observed simultaneously in a single sweep. In ΔKPQ-mutant macropatch currents, the inward deflections were short, with an amplitude of ~10 to 25 pA in most patches in our experiment. In Fig. 1A, the averaged peak inward current was −12.4 pA, suggesting that there were 15 functioning channels in the patch (see *Materials and Methods*). After the initial fast inward deflection, late openings were commonly observed in the ΔKPQ Na^+ channel. As has also been widely observed in native cardiac myocytes, the late openings were composed of two types of activities, i.e., burst openings and isolated openings. Under the effect of 1 μM pilsicainide (Fig. 1B) or lidocaine (Fig. 1C), however, late openings were rare in appearance and short in duration. When pilsicainide or lidocaine was present in the pipette solution, prominent decreases in the probability of long-lasting bursts were evident, as indicated by a decrease in the

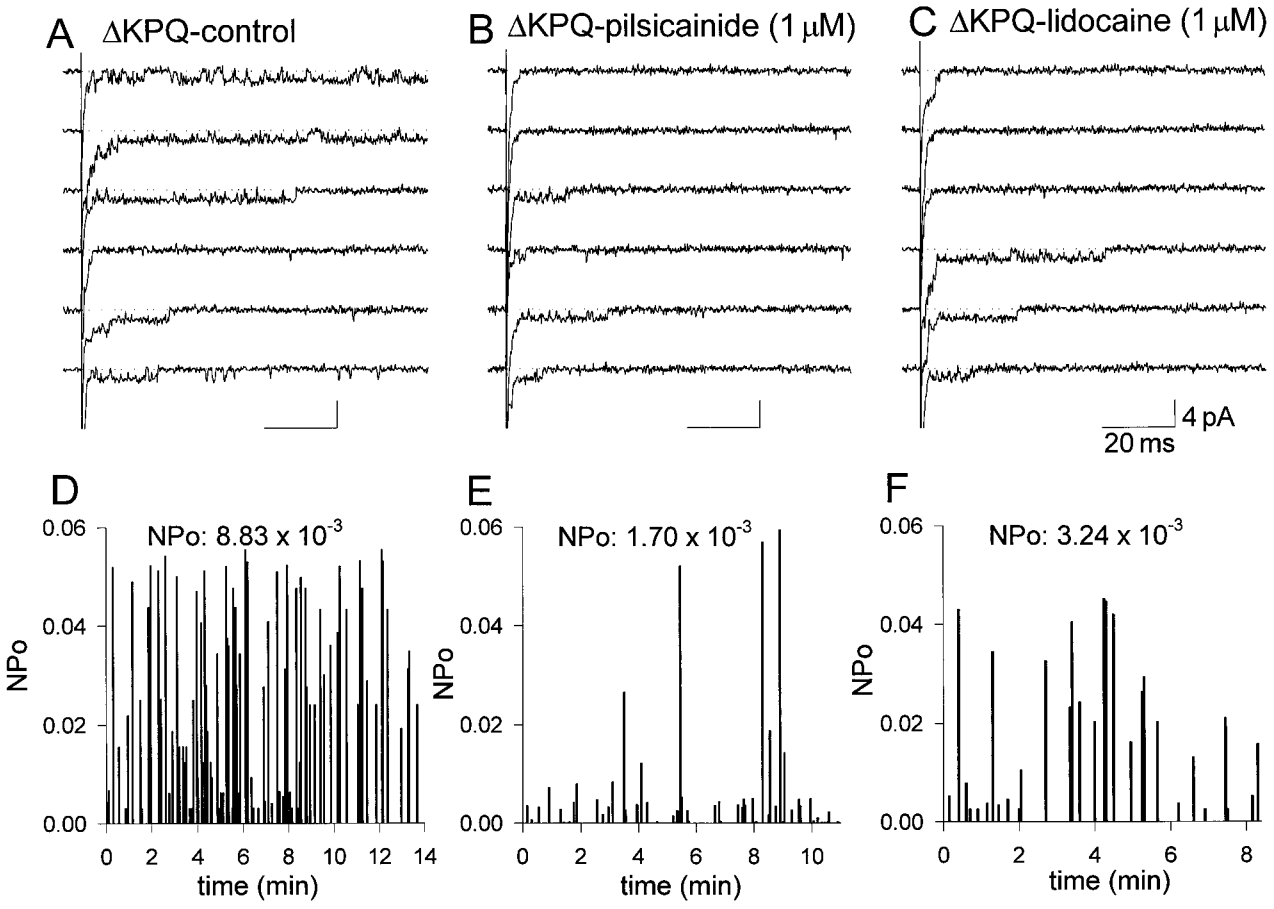

Fig. 1. Single-channel activities in ΔKPQ-mutant channels with or without local anesthetics (A–C) and diagrams of their open probability in late currents (D–F). Six consecutive traces were evoked by a test potential of 0 mV from a holding potential of −120 mV at a frequency of 0.5 Hz. Each test pulse lasted 195 ms, and current traces for the initial 100 ms are shown. Channel openings are downward deflections in the current traces. The start of the test pulse is coincident with the residual uncorrected capacitive current. The initial peak currents, whose ensemble average of traces was −12.4 pA (411 sweeps) in control conditions (A), −10.6 pA (330 sweeps) in the presence of 1 μM pilsicainide (B), and −13.1 pA (256 sweeps) in the presence of 1 μM lidocaine (C), are all off scale. Diagrams of open probability (NPo) in late currents were constructed for channel openings that excluded those for the initial 10 ms after the onset of depolarization. Mean NPo values calculated through the observation periods are indicated (D–F).

number of sweeps with openings between 10 and 95 ms (Fig. 1, D–F). In these examples, the mean NPo obtained in late activities starting from 10 ms after depolarization to the end of the pulse was 8.83×10^{-3} in the absence of drug (Fig. 1D), 1.70×10^{-3} in the presence of 1 μM pilsicainide (Fig. 1E), and 3.24×10^{-3} in the presence of 1 μM lidocaine (Fig. 1F). A similar reduction in NPo in late openings was observed in seven patches with 1 μM pilsicainide (NPo = $2.39 \times 10^{-3} \pm 1.10 \times 10^{-3}$) and in six patches with 1 μM lidocaine (NPo = $3.86 \times 10^{-3} \pm 1.63 \times 10^{-3}$), compared with NPo without drug (NPo = $9.80 \times 10^{-3} \pm 2.60 \times 10^{-3}$; $n = 9$).

We have attempted to identify changes in the open and closed times of bursting activity induced by pilsicainide and lidocaine. If pilsicainide acts as an open channel blocker and lidocaine acts as an inactivation channel blocker, we expect to see differences in the distributions of open time and closed time under the effects of these agents. Because we discarded overlapping bursting events, and we never encountered apparent bursting events after a long intervening period (>20 ms), we reasoned that one bursting activity was derived from a single channel but not from the mixed activities of multiple channels. Figure 2 shows a comparison of the open time during burst activities with or without the actions of channel blockers. In control conditions, open times (Fig. 2A) were best fitted with a single exponential ($\tau = 5.07$ ms). Because it was technically difficult to apply lidocaine and pilsicainide to the same recording patch with an interposed wash-out period, open-time histograms of bursts from seven cell-attached macropatches in control conditions, five cell-attached macropatches in the presence of 1 μM pilsicainide, and five cell-attached macropatches in the presence of lidocaine were all accumulated and then fitted (Fig. 2). This improved our ability to detect exact time constants within the histograms. To increase our confidence that combining the data did not produce a heterogeneous population of channels, we compared single-channel amplitudes in the control and under the effects of drugs during the burst. The single-channel amplitude was -1.60 ± 0.08 pA at 0 mV in the control condition, -1.56 ± 0.10 pA with 1 μM pilsicainide, and -1.55 ± 0.08 pA with 1 μM lidocaine in the presence of drugs in the pipette for burst openings (data not shown). Under the effect of 1 μM pilsicainide, the time constant ($\tau = 4.80$ ms) for the open-time fitted curve was slightly shortened, and it was comparatively short ($\tau = 3.64$ ms) for the same concentration of lidocaine (Fig. 1C). A small reduction in open times in the presence of pilsicainide or lidocaine can be seen, which is suggestive of an open channel block of bursting activity by both drugs. However, the small changes in open times cannot account for the large decrease in NPo for the late current observed in Fig. 1. We then analyzed the closed times from the same populations of activities as shown in Fig. 2, which were similarly accumulated from multiple patches (seven patches for control, five patches in the presence of pilsicainide, and five patches in the presence of lidocaine). Closed times were best fitted with two exponentials in the control condition (Fig. 3A), and during exposure to pilsicainide or lidocaine (Fig. 3, B and C), which were all fitted better than by one exponential. The fast time constants ($\tau_f = 0.50$ ms in the presence of pilsicainide and 0.44 ms in the presence of lidocaine) were prolonged when compared with that in the control condition ($\tau_f = 0.33$ ms). The slow time constants ($\tau_s = 1.87$ ms with pilsicainide and 1.63 ms with lidocaine) were similarly prolonged when compared with that in the control condition ($\tau_s = 1.41$ ms). The relatively small difference in the time constants in the closed time distribution of burst openings in the presence of pilsicainide or lidocaine could not account for the difference in action of the drugs on the NPo in the late openings.

To identify the contribution of bursting activity toward the changes in the open probability, we then evaluated burst

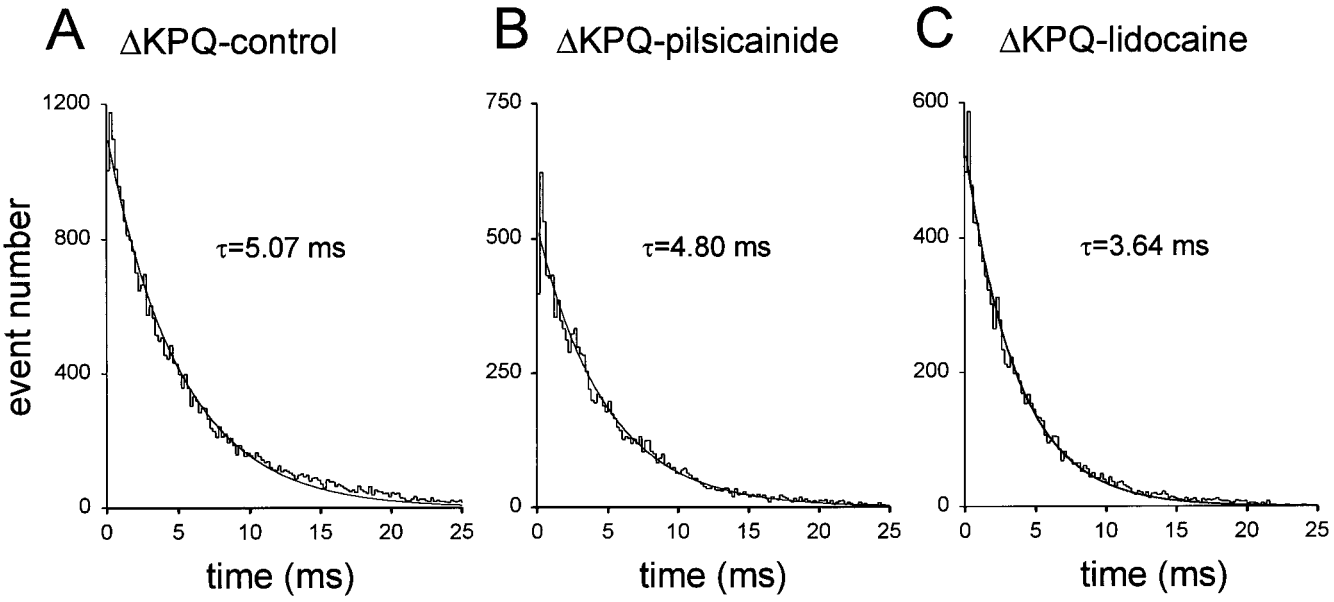

Fig. 2. Histogram analysis of open times in control conditions (A) and in the presence of 1 μM pilsicainide (B) or 1 μM lidocaine (C). Open-time duration was measured from idealized recordings during burst openings at 0 mV. Events during the initial 10 ms were excluded from the result. The histograms were plotted with a bin width of 0.2 ms, in which the mean open time corresponds to the time constant of the smooth curve obtained by fitting the histogram to single-exponential decay functions with the indicated time constant (τ). Complications arising from overlapping openings in the analysis of burst openings were avoided by discarding overlapping events. Mean open times in the ΔKPQ-mutant channel in the control and in the presence of 1 μM pilsicainide and of 1 μM lidocaine were 5.07 ms (seven patches), 4.80 ms (five patches), and 3.64 ms (five patches), respectively.

duration under the effects of drugs by means of burst-duration histograms as shown in Fig. 4. The histograms were constructed by typical three-patch records that represented the control condition (Fig. 4A), the condition under the effect of pilsicainide (Fig. 4B), and that under the effect of lidocaine (Fig. 4C), respectively. Burst duration was exponentially distributed with a mean of 34.0 ± 3.8 ms (n = 6) in the control, 18.2 ± 4.4 ms (n = 5) with pilsicainide, and 26.7 ± 3.1 ms with lidocaine. Mean bursting times were reduced to 55% of the control with pilsicainide and 75% of the control with lidocaine at the test potential of 0 mV.

Complete experiments in which the effects of pilsicainide and lidocaine were examined on the initial peak current and the late current simultaneously would be necessary to obtain the selectivity of the drugs in blocking the late currents. Instead of dialyzing the pipette solution with a solution containing the drugs after GΩ seal formation and control current recordings, which was technically difficult, we applied the pipette solution containing pilsicainide or lidocaine gently from the open side of the pipette after filling the pipette by dipping it into a small beaker containing the solution without drugs (see *Materials and Methods* and Fig. 5A). This technique allowed the serial observation of both the transient fast Na^+ current and the late openings in proportion as a bulk backfilled drug solution diffused to the patched membrane. The individual current traces yielded both the initial peak current (I_{Na}) and the NPo derived from the late currents (Fig. 5, B and C). For most of the traces in our study with a very high concentration of pilsicainide (100–500 μM), I_{Na} was unchanged for the initial 90 to 120 s after the GΩ seal formation and started decreasing in peak amplitude thereafter. The current amplitude usually reached stable conditions within 4 min after the GΩ formation, as shown for a typical patch in Fig. 5D. We therefore considered I_{Na} and NPo recorded during the initial 1 min to be values without drug action and the I_{Na} and NPo recorded after 4 min of GΩ formation to be the same values under the drug action. In six experiments, pairs of data sets were obtained during control and 1-μM-pilsicainide exposure on the same patch as shown in Fig. 5, E–G. They were also obtained for control and 1 μM lidocaine exposures in the same fashion in the same number of experiments. The I_{Na} was only slightly decreased by 1 μM pilsicainide (by 6%) or by 1 μM lidocaine (by 4%), as shown in Fig. 5E, but NPo values obtained from late channel activities (burst and isolated openings) were markedly decreased by the drugs, particularly 1 μM pilsicainide (Fig. 5F). The NPo of late current was halved by 1 μM pilsicainide, and this reduction was significantly large compared with the decrease induced by 1 μM lidocaine. Because the changes in I_{Na} and late current NPo were recorded from the same trace, the ratio of the changes in NPo to the I_{Na} would be expected to show a selectivity of inhibition of the late current by the drugs. Figure 5G shows the NPo value over the I_{Na} under the effects of these drugs. The reduction in the late NPo was very large with 1 μM pilsicainide compared with the decrease in I_{Na}, where NPo/I_{Na} was ~0.4, suggesting that pilsicainide selectively inhibited late openings of ΔKPQ Na^+ channels without preferably affecting the I_{Na}. Judged from the NPo/I_{Na} values for pilsicainide and lidocaine, the blocking selectivity of pilsicainide in the late current might be twice as high as that of lidocaine.

Another measure of blocking selectivity in the late currents includes the drug-blocking ratio dependent on the opening type. Because late currents in the ΔKPQ Na^+ channels were composed of two types of openings, i.e., burst openings and isolated openings, an examination of possible selective blocking of burst openings or isolated openings would be of interest. By a simple algorithm that discriminated burst openings from isolated openings, we were able to obtain three sets of data from an individual trace, which included I_{Na}, the open

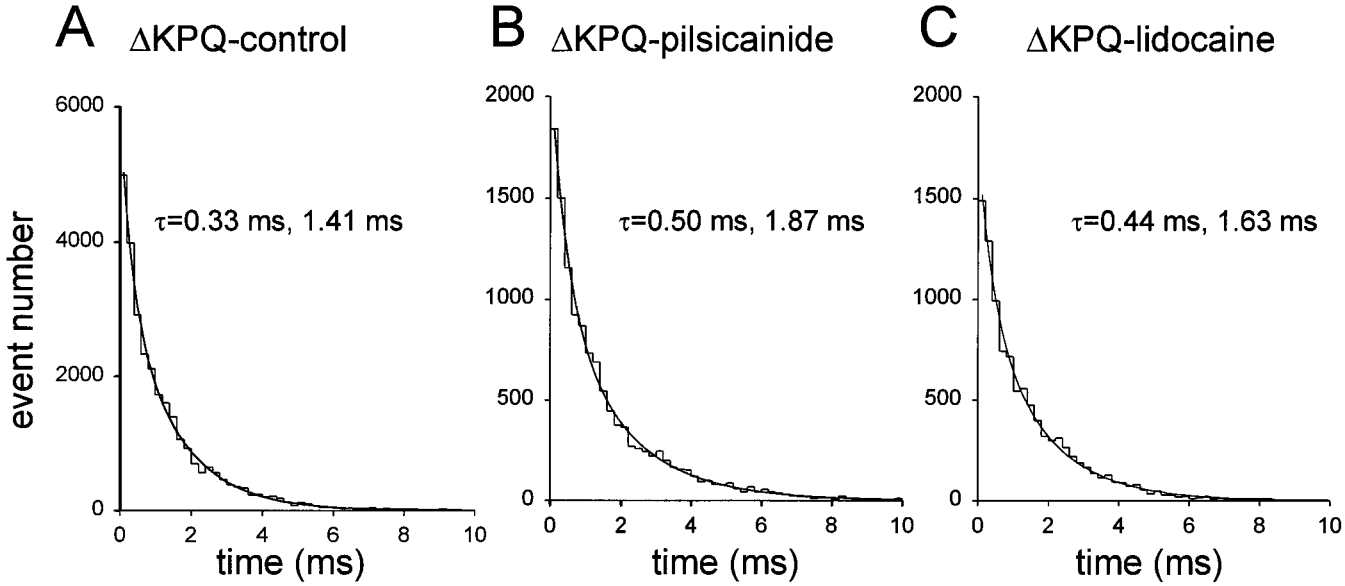

Fig. 3. Histogram analysis of closed times in control conditions (A) and in the presence of 1 μM pilsicainide (B) or 1 μM lidocaine (C). Closed-time durations during the burst openings were measured from the same data set as in Fig. 2 at 0 mV with the use of the same open/closed tables. The histograms were plotted with a bin width of 0.2 ms and fitted by the sum of two exponentials in either control conditions (A) or in the presence of pilsicainide (B) or lidocaine (C), with the indicated time constant (τ). Time constants for fast (τ_f) and slow (τ_s) components in control conditions were 0.33 and 1.41 ms, respectively (n = 7 patches). These value were 0.50 ms (τ_f) and 1.87 ms (τ_s) in the presence of 1 μM pilsicainide (n = 5) and were 0.44 ms (τ_f) and 1.63 ms (τ_s) with 1 μM lidocaine (n = 5).

probability derived only from bursting open ($_b$NPo), and the open probability derived only from isolated openings ($_i$NPo). By the same drug delivery system shown in Fig. 5, changes in $_b$NPo and $_i$NPo by pilsicainide were demonstrated separately, as in Fig. 6B. By this analysis, the changes in NPo of overall late currents were sorted into changes in $_b$NPo and $_i$NPo accordingly. The difference in the reduction in $_b$NPo induced by pilsicainide or by lidocaine was remarkable; pilsicainide strongly decreased $_b$NPo, whereas lidocaine only slightly suppressed it. On the other hand, 1 μM pilsicainide was practically ineffective in the suppression of isolated openings ($_i$NPo), whereas the reduction in $_i$NPo by lidocaine was significantly larger than that of pilsicainide (Fig. 6E). Because $_b$NPo/$_i$NPo without any drug present should reflect the contribution ratio of bursting activity to the entire late current, $_b$NPo/$_i$NPo under the effects of drugs would account for the residual late channel activities exposed to blockers. In untreated ΔKPQ Na^+ channels, the contribution ratio of $_b$NPo and $_i$NPo to the late currents was ~6:1 (Fig. 6F). With the effects of pilsicainide, the current component carried by $_b$NPo and $_i$NPo was nearly identical. The contribution of $_b$NPo to the late current was more than that of the control when lidocaine was applied.

Discussion

In this study, we have demonstrated for the first time that low concentrations of pilsicainide, an open channel blocker of Na^+ channels, selectively suppressed the late currents in ΔKPQ-mutant Na^+ channels by shortening the burst duration. By a combination of the macropatch method and a unique drug delivery system to the patched membrane, we were able to evaluate the changes in the initial I_{Na} simultaneously with the changes in burst and isolated openings in the same patch. During the late currents produced in the ΔKPQ-mutant Na^+ channels, burst openings contributed to the open probability at a value six times that of isolated openings. Pilsicainide selectively suppressed the contribution of burst openings to the late currents, in great contrast to lidocaine, which nonselectively inhibited both the burst and isolated openings during the late currents.

Preferential Suppression of Late Currents. A dominant feature of the ΔKPQ-mutant Na^+ channel is the inclination to open more frequently, resulting in producing late currents. The mutant channel may enter a conformation during the altered gating mode that has a higher affinity for local anesthetics. We speculate that pilsicainide, a class Ic antiarrhythmic agent that has high affinity to the open channel, preferably interacts and inhibits the late currents. On the other hand, lidocaine, a class Ib antiarrhythmic agent with high affinity for the inactivated channel, probably exerts a moderate effect on the late current, because this mutant is deficient in normal inactivation processes, and lidocaine acts to block inactivated rather than open channels (Bean et al., 1983; Bennett et al., 1995a). Therefore, we examined the possible differential effect of an open channel blocker of Na^+ channels on the late currents. As clearly demonstrated in Fig. 6, pilsicainide (1 μM) drastically inhibited the late current with only a small (~5%) reduction in the initial current. Because the mean open time (Fig. 2) and the mean closed times (Fig. 3) during the burst were not remarkably changed and the burst duration was markedly shortened, it is reasonable to speculate that pilsicainide binds to the mutant channels when the bursting late openings are repeated, or the repetitive openings increase the probability for pilsicainide to bind to the channel. This speculation may be consistent with previous findings that pilsicainide acts as a "slow" drug to the cardiac Na^+ channel (Inomata et al., 1989; Kodama et al., 1999).

On the other hand, the effects of lidocaine on the late currents are rather complicated. Lidocaine block of native Na^+ channels is known to depend on the state of the Na^+ channel and is more pronounced for channels in the inactivated state (Hondeghem and Katzung, 1977; Starmer and Courtney, 1986). Bennett et al. (1995a) proposed that the ΔKPQ mutation caused an alteration in gating mode in which the channels reopen from the inactivated state, pro-

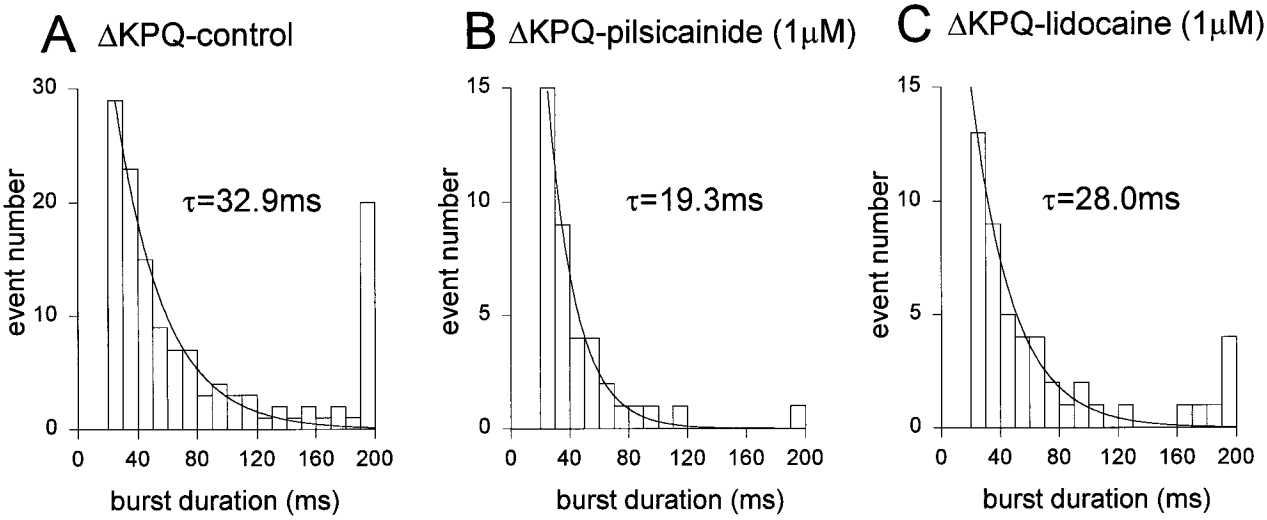

Fig. 4. Histograms of burst duration in control conditions (A) and in the presence of pilsicainide (B) or lidocaine (C). Data are from three representative patches for bursts longer than 20 ms. Sweeps in which the channels remained open at the end of the sweep are shown in the last bin. Solid lines are the single-exponential fit to the data, predicting the mean burst duration as indicated. Grouped data of mean burst duration at 0 mV in control conditions and in the presence of 1 μM pilsicainide or 1 μM lidocaine were 34.0 $\pm$ 3.8 ms (six patches), 18.2 $\pm$ 4.4 ms (five patches), and 26.7 $\pm$ 3.1 ms (five patches), respectively.

ducing burst openings during maintained depolarization. In this case, the ΔKPQ channel that makes transitions between the inactivated state and a mode of gating in which bursts of activity occur for a prolonged period could likely be blocked by lidocaine, which has a higher affinity for the inactivated state of the channel. However, no remarkable inhibitory action of lidocaine on the burst duration (Fig. 4) or open probability produced by burst openings (Fig. 6D) was observed in this study. Instead, lidocaine significantly decreased the isolated openings ($_i$NPo) compared with pilsicainide. In addition, 1 μM lidocaine had a rather small effect on the mean open and closed times during the burst, although the mean open times were shortened by ~30%.

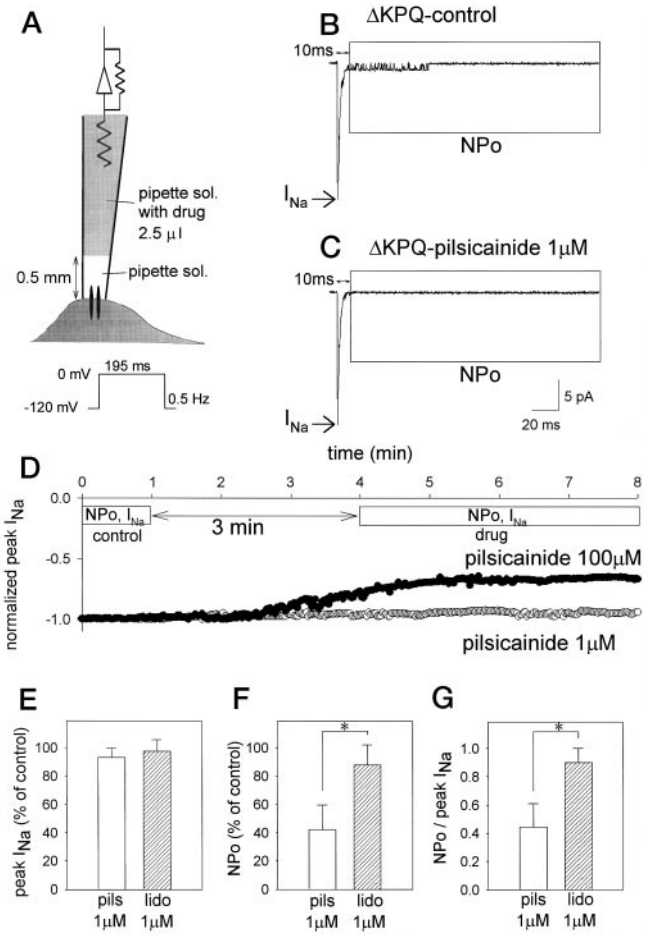

Fig. 5. Comparison of the changes in the initial peak current (I_{Na}) and the open probability for late openings (NPo). A, a drug delivery system to the patched membrane and the voltage protocol are illustrated (see *Materials and Methods*). I_{Na} and NPo were measured in individual current traces in control conditions (B) and in the presence of pilsicainide (C), and they were averaged and normalized for the effects of drugs; the value of 100% was assigned as the control value. D, samples of changes in normalized I_{Na} in accordance with an application of 1 μM pilsicainide or 100 μM pilsicainide to the pipette solution (see *Materials and Methods*) were plotted against time after GΩ seal formation. A simple illustration of the backfilled pipette solution with an anesthetic drug is shown in the inset. E, normalized I_{Na} after the application of 1 μM pilsicainide (n = 5) and 1 μM lidocaine (n = 5). The mean tonic block of the I_{Na} was <5% when induced by either pilsicainide or lidocaine. F, normalized NPo of late currents showing the effects of 1 μM pilsicainide and 1 μM lidocaine. Pilsicainide was significantly (P = .002) effective in suppressing the late current compared with lidocaine. G, summary data for ratio of the late-current NPo to I_{Na} when the drugs were applied, indicating that the NPo/I_{Na} ratio was significantly smaller due to the effects of pilsicainide when compared with those of lidocaine. *P < .05.

$$C \rightarrow O \rightarrow I$$

Scheme 1.

It is widely accepted that WT Na^+ channels open once at most depolarized potentials; i.e., the fast inactivated state is absorbing, as illustrated in Scheme 1. Because the initial transient current of the ΔKPQ Na^+ channel was virtually not inhibited by 1 μM pilsicainide or lidocaine (Fig. 5E), it is postulated that the relatively low concentration of these agents selectively bind to the ΔKPQ channel after the channel experiences the first openings. Therefore, taken together,

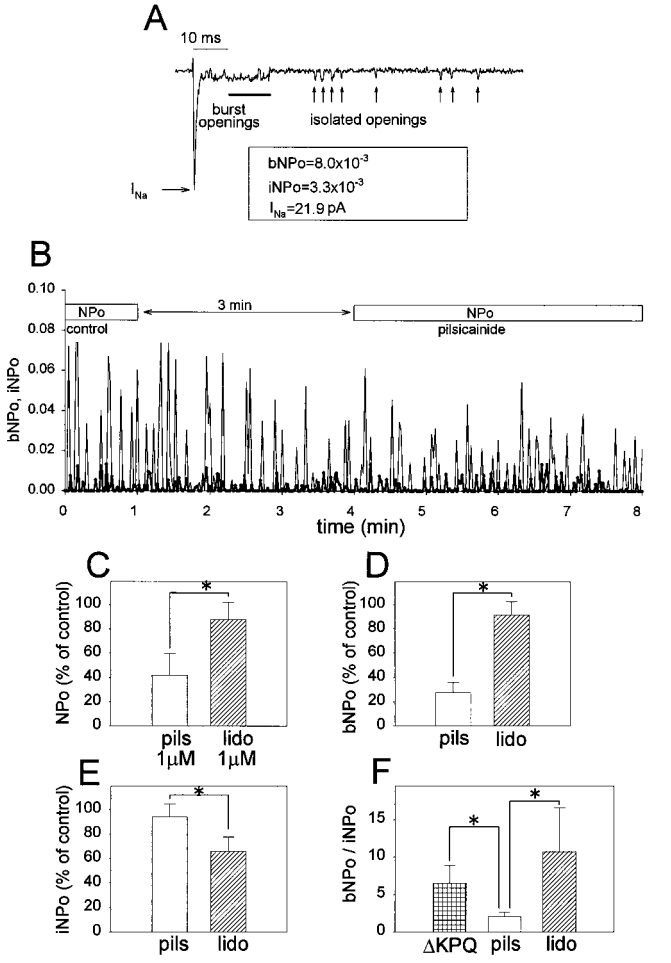

Fig. 6. Selectivity of blocks of burst openings and isolated openings. A, a representative macropatch current trace, showing both burst and isolated openings. Open probability (NPo) was calculated independently of the opening types: NPo derived only from burst openings ($_b$NPo), NPo derived only from isolated openings ($_i$NPo), and the I_{Na} in this particular sweep are indicated. B, a diagram demonstrating changes in $_b$NPo (without symbols) and $_i$NPo (with ●). Mean $_b$NPo and $_i$NPo for the initial 1 min (control) and during the effects of drugs (for a duration after a 4-min interval after the formation of the GΩ seal) were calculated for further analyses. In this particular patch, $_b$NPo and $_i$NPo in control conditions were 1.38×10^{-2} and 1.85×10^{-3}, whereas they were 6.31×10^{-3} and 1.57×10^{-3} under the effect of 1 μM pilsicainide in the pipette solution. C, normalized NPo of overall late currents, which are identical with Fig. 5E. D, normalized $_b$NPo during effects of drugs. $_b$NPo was greatly reduced by 1 μM pilsicainide, and the reduction was significantly larger than that induced by lidocaine. E, normalized $_i$NPo during the effects of the drugs. C, D, and E were obtained from identical data sets. F, the ratio of appearance of burst openings to isolated openings was expressed as the ratio of the open probability for each of these ($_b$NPo/$_i$NPo) under control conditions (ΔKPQ) and under the effects of drugs [1 μM pilsicainide (pils) or lidocaine (lido)]. *P < .05.

we speculate that ΔKPQ channels cause altered gating modes in which the mutant channels reopen from the inactivated state to produce isolated openings, as shown in Scheme 2. This is the simplest model possible for the selective block of the isolated openings by lidocaine based on the evidence presented herein. The proposed kinetic model for the burst openings and the block by pilsicainide is illustrated in Scheme 3. It is likely that a high probability for the channel to stay in the burst-openings (O_b) state, with delayed ($O_b \rightarrow I$) transitions, is favored by pilsicainide, an open channel blocker. The reversal of the blocked state (O_bP) to the burst-openings state (O_b) could be responsible for a small decrease in the mean open time induced by pilsicainide.

Therapeutic Implications. There is some clinical evidence that LQT3 patients are likely to shorten their QT intervals to a greater extent than healthy control subjects during a physiologically induced increase in heart rate (Schwartz et al., 1995b), probably because of the delayed recovery from the inactivation in the rate-dependent block of the late current in mutant Na^+ channels at tachycardia. This is in agreement with findings that most cardiac arrests occur during sleep or at rest among LQT3 patients (Schwartz et al., 1995b). Therefore, it is probably therapeutically more important for LQT3 patients to control their QT intervals, particularly under low heart rate conditions, rather than the arrhythmias by themselves. In this experiment, we used a stimulation frequency of 0.5 Hz to examine the tonic blocking action of pilsicainide and lidocaine on late Na^+ currents. We did not apply higher stimulation frequencies to test actions of pilsicainide or lidocaine, because it is speculated that the late currents could be blocked use dependently at high stimulation frequencies without drugs.

For the purpose of reducing the late currents in the mutant Na^+ channels, lidocaine and mexiletine have been examined for their actions (An et al., 1996; Wang et al., 1997). For instance, An et al. (1996) demonstrated the suppression of late-opening Na^+ channels by a high concentration (100 μM) of lidocaine in the ΔKPQ-mutant channels. However, lidocaine of this concentration blocked the initial peak current of ΔKPQ channels by ~30%, and the block was greater in ΔKPQ-Na^+ channels than in the WT Na^+ channels; lidocaine may not be the optimal drug to suppress the late current selectively. In contrast, the EC_{50} of pilsicainide to block late currents in ΔKPQ mutants is speculated to be <1 μM by the studies shown in Fig. 5F. Moreover, unlike the anticholinergic effect of disopyramide (a class Ic antiarrhythmic agent), that of pilsicainide is negligibly small at therapeutic plasma concentrations (3–10 μM) (Inomata et al., 1989). Therefore, the high selectivity of pilsicainide to block the late currents (cf. Fig. 5, E and F) suggests an additional choice for the pharmacological treatment of LQT3 patients.

$$Cn \rightarrow O_i \Leftrightarrow I \rightarrow IL$$

Scheme 2.

$$Cn \Leftrightarrow O_b \rightarrow O_bP$$
$$\downarrow$$
$$I$$

Scheme 3.

Advantages and Limitations of the Study. An important advantage of the method in this study was the use of cell-attached macropatches in recording mutant Na^+ channels. Because we were able to record and compare the transient initial current and the late current from the same patch, a direct comparison of their suppression was successful. We believe that this method increases the reliability of assessing a drug's selectivity for blocking the late currents. In addition, we expressed the mutant channel in a mammalian cell line (CHW cell), which is suitable for examining the effect of antiarrhythmic drugs. Because evidence has been provided that local anesthetics bind to a site that is within the channel pore but accessible only from the intracellular side of the cell (Strichartz, 1977; Cahalan and Almers, 1979), plasma cell membranes in a mammalian cell line would provide a more pertinent pathway for evaluation of drug actions on the expressed Na^+ channels than *Xenopus* oocytes.

Limitations due to issues regarding the function of subunits must be considered. We have only expressed the α-subunit of ΔKPQ-mutant channels in this study. The β-subunit contributes to the rapid inactivation of Na^+ channels (Isom et al., 1994) in which the rapid component of inactivation may play a role in the block. According to a study by Makielski et al. (1996), the β_1-subunit affects the function of the Na^+ channel expressed in oocytes by decreasing the tonic and phasic lidocaine block. Further studies are required in which both the α- and β-subunits of ΔKPQ-mutant Na^+ channels are expressed. Caution must be used, of course, when directly extrapolating our data obtained in in vitro experimental settings only from heterologously expressed mutant channels without exploring the possible effects of these drugs in vivo or on other ion channels or on the autonomic control of cardiac excitability in detail. Moreover, particular care must be exercised with the use of pilsicainide, as a class Ic drug, because of potential adverse effects on postinfarct LQT3 patients (CAST investigators, 1989).

Summary. In conclusion, we demonstrated that pilsicainide, an open channel blocker of Na^+ channels, is capable of selectively blocking the late openings in ΔKPQ-mutant channels. The most striking action of pilsicainide is to preferentially block burst openings. Our results provide a new therapeutic insight into management of late currents in a genetic disorder of Na^+ channels, introducing an indication of a burst-opening blocker or isolated-opening blocker, depending on phenotypes based on the molecular properties of the channels.

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