Mechanism of lidocaine block of late current in long Q-T mutant Na\(^+\) channels

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Dumaine, R., and G. E. Kirsch. Mechanism of lidocaine block of late current in long Q-T mutant Na\(^+\) channels. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H477–H487, 1998.—Inherited long Q-T syndrome is a ventricular arrhythmia associated with delayed repolarization and the risk of sudden death. The chromosome 3-linked form of the disease (LQT3) is associated with mutations in the cardiac Na\(^+\) channel (N1325S or R1644H; or deletion of residues 1,505–1,507, ΔKPQ) that increase late inward currents and may cause delayed repolarization. Late currents arise from dispersed reopenings (N1325S and R1644H) or from reopenings combined with prolonged bursts (ΔKPQ). Therefore, we tested whether lidocaine blockade of late current varied among the different LQT3 mutant channels. We found that lidocaine preferentially blocked late over peak current and that the blockade was equally effective in all three channels, expressed in Xenopus oocytes. Lidocaine inhibited both dispersed reopenings and bursting in single channels without affecting mean open times. In the absence of drug, inactivating prepulses inhibited bursting but did not change the mean open times. We suggest that lidocaine block of late current in LQT3 channels acts via a common mechanism involving stabilization of inactivation. Therefore, blockers that target the inactivated state may be effective therapeutic agents in all three biophysical phenotypes of LQT3.

cardiac arrhythmia; human heart; Romano-Ward syndrome; SCN5A

IN THE LONG Q-T SYNDROME prolongation of the Q-T interval of the electrocardiogram is associated with polymorphic ventricular tachycardia (torsades de pointes), cardiac arrest, and sudden death (30). The Romano-Ward form of the disease is inherited in an autosomal dominant pattern and is genetically heterogeneous. Linkage analysis has identified four disease-related loci, at least three of which are known to involve genes that encode ion channels. LQT1 (chromosome 11-linked; Ref. 34) and LQT2 (chromosome 7-linked; Refs. 10, 27) are associated with mutations in voltage-gated potassium channel genes (KVLQT1 and HERC, respectively), and LQT3 (chromosome 3-linked; Ref. 35) is associated with mutations in SCN5A, a gene that encodes the cardiac Na\(^+\) channel.

The balance between inward, depolarizing and outward, repolarizing currents through ion channels regulates ventricular repolarization. Thus a decrease in outward K\(^+\) currents or an increase in inward Ca\(^{2+}\) or Na\(^+\) currents has the potential to lengthen the Q-T interval and induce early afterdepolarizations (1, 11, 20). Because membrane resistance during the action potential plateau is very high, small changes in late ionic currents can markedly alter the action potential waveform and the Q-T interval (14, 26). Late Na\(^+\) current that persists after fast inactivation has reached a steady state has been shown to prolong the action potential plateau in animal models (3, 9, 16, 21). In heterologous expression systems, human LQT3 mutant Na\(^+\) channels exhibit increased levels of late Na\(^+\) current (6, 13, 32) that may be directly responsible for the disease.

We previously reported (13) that the LQT3 mutations ΔKPQ (deletion of a lysine, proline, and glutamine at positions 1,505–1,507), R1644H (arginine-to-histidine substitution), and N1325S (asparagine-to-serine substitution) cause an increase in late current by promoting two types of inactivation abnormalities: short reopenings indicative of an accelerated exit from the inactivated state (i.e., reduced stability) and prolonged bursting, indicative of a switch from a normal to a bursting (i.e., noninactivating) mode of gating. Na\(^+\)-channel blockers, such as class I antiarrhythmics, are potentially useful therapeutic agents. However, drugs that act by promoting inactivation might not be as effective in blocking the noninactivating mode of channel activity as in correcting defects involving reduced stability of the inactivated state. In the present study, we chose lidocaine as a prototypic inactivation-promoting class Ib antiarrhythmic to address this question. Previous studies (2, 13, 33) showed that the late current is more sensitive than the peak current to block by class Ib antiarrhythmics, but the mechanism is unclear, particularly in view of previous observations that noninactivating mutant channels have reduced sensitivity to block by lidocaine (5).

In the present study, we found that the frequency of occurrence of bursting activity was suppressed by inactivating prepulses. Moreover, the effectiveness of lidocaine in blocking late current was enhanced at depolarized holding potentials. Lidocaine, which potentiates the inactivated state, reduced the frequency of occurrence of both bursts and dispersed openings by increasing the closed-time duration between the events but did not change the mean open times. Our results indicate that lidocaine exerts its action on the late current by stabilizing inactivation in LQT3 mutant Na\(^+\) channels, thereby reducing the probability of occurrence of the dispersed and bursting activity nonselectively.

METHODS

Na\(^+\)-channel cDNA and mutagenesis. The human heart Na\(^+\)-channel cDNA (hH1a, expression plasmid of SCN5A) was the same as previously described (17). The full-length cDNA was cloned into the pGEM3 plasmid vector (Promega, Madison, WI). The final SCN5A expression construct contains...
cDNA sequence from nucleotide (nt) 123 to nt 6,333 of the published SCN5A cDNA sequence (15).

LQT3 is associated with three defects in the primary structure of the cardiac Na⁺ channel (35): a substitution of serine for asparagine at position 1,325 (in the intracellular linker between transmembrane segments S4 and S5 of domain III), a substitution of histidine for arginine at position 1,644 (at the intracellular end of transmembrane segment S4 in domain IV), or a three-residue deletion of lysine-proline-serine for asparagine at position 1,505–1,507. The mutant channels resulting from the expression of these three constructs are denoted in the text by single-letter amino acid abbreviations: N/S, R/H, and ΔKPQ, respectively. The nonmutated (wild type) channel is denoted WT. LQT3 mutations were produced by site-directed, polymerase chain reaction-based mutagenesis using the megaprimer method (28). All three mutant constructs were verified by DNA sequencing.

RNA transcription and oocyte injection. DNA constructs were linearized by digestion with Hind III, and in vitro transcription with T7 RNA polymerase was performed using the Message Machine kit (Ambion, Austin, TX). The amount of cRNA product was quantified by incorporation of trace amounts of [32P]UTP in the synthesis mixture, and the integrity of the cRNA was determined using a denaturing agarose gel stained with ethidium bromide. cRNA was resuspended in 0.1 M KCl at a concentration of 250 ng/ml and stored at −80°C. Before use, cRNA was diluted to the desired concentration (generally 1–10 pg/ml). Stage V-VI Xenopus oocytes were defolliculated by collagenase treatment (2 mg/ml for 1.5 h) in a nominally Ca²⁺-free buffer solution containing (in mM) 82.5 NaCl, 2.5 KCl, 1 MgCl₂, and 5 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) with 100 mg/ml gentamicin, pH 7.6. The oocytes were injected with 46 ng/ml of cRNA solution (in 0.1 M KCl) and incubated at 19°C in culture medium containing (in mM) 100 NaCl, 2 KCl, 1.8 CaCl₂, 1 MgCl₂, 5 HEPES, and 2.5 pyruvic acid with 100 mg/ml gentamicin, pH 7.6. Electrophysiological measurements were made 5–10 days after cRNA injection. The amount of cRNA injected was varied according to the purpose of the experiment. The amount of cRNA was adjusted to give peak whole cell currents in domain III, or a three-residue deletion of lysine-proline-glutamine from positions 1,506–1,507. The mutant channels resulting from the expression of these three constructs are denoted as follows: N/S, R/H, and ΔKPQ, respectively. The nonmutated (wild type) channel is denoted WT. LQT3 mutations were produced by site-directed, polymerase chain reaction-based mutagenesis using the megaprimer method (28). All three mutant constructs were verified by DNA sequencing.

Electrophysiological measurements were performed on the isolated oocyte membrane by means of the two-microelectrode voltage clamp technique. The oocytes were bathed in drug solution for an equilibration period of 6 min during which the cells were pulsed at 15-s intervals. Figure 1A compares a control record obtained before drug application with that obtained after equilibration of resting ΔKPQ channels with bath-applied lidocaine at 50 μM. This concentration typically reduced peak current by <10%, indicative of the relatively low sensitivity of resting channels to drug block. As shown by the filled symbols in the dose-response relationship (Fig. 1D), similar levels of block were observed in all four types of channels such that tonic block was negligible at drug concentrations <100 μM. Shifting the holding potential to −100 mV, however, resulted in an apparent increase in tonic block that was particularly evident at higher concentrations.
As discussed below, the amount of tonic block observed at higher concentrations and more depolarized holding potentials may not provide an accurate estimate of resting channel block caused by drug-enhanced inactivation. Nonetheless, the results suggest that when held in the resting state, all of the channels were highly resistant to drug occupancy.

We measured phasic block using a three-pulse protocol (Fig. 1B). A long conditioning pulse (0-mV amplitude, 960-ms duration) allowed drug equilibration with channels in the inactivated state. Next, a brief prepulse (−140 mV, 10 ms) allowed unblocked channels to recover from inactivation. The amount of block was determined from measurement of peak current during a brief test pulse (0 mV, 10 ms) that maximally activated the channels. Figure 1B shows superimposed ΔKPQ test pulse current records under control conditions and after equilibration with 50 µM lidocaine. An
average 55% inhibition of peak current obtained after preconditioning with a long depolarizing pulse indicated a much stronger block of the inactivated than the resting state. Figure 1D shows that this effect was due to a leftward shift of the dose-response relationship such that substantial phasic block (>20%) occurred at a concentration (10 µM) that was ineffective in producing tonic block. Phasic block showed an apparent dissociation constant ($K_d$) of $32 \pm 1$, $39 \pm 3$, $25 \pm 3$, and $33 \pm 5$ µM for WT, ΔKPQ, RH and N/S, respectively. These values were not significantly different by ANOVA test. Our results showed that lidocaine block of channels in the inactivated states was comparable between WT and LQT3 channels and suggest that the affinity for lidocaine was not modified by the mutations.

We next compared the effectiveness of lidocaine in blocking the late current in LQT3 channels (late current in WT channels was too small to resolve lidocaine block). Late current block was measured from inhibition of currents evoked by 500-ms test pulses to 0 mV from a holding potential of $-100$ mV. Isochronal current amplitudes at 300 ms were compared before and after drug application. Figure 1C illustrates typical results obtained in ΔKPQ channels in 50 µM lidocaine. An average value of 36% block obtained under these conditions was slightly less than the amount of phasic block at the same concentration. Nonspecific currents were corrected by subtraction of current evoked by the same pulse protocol after application of 100 µM tetrodotoxin (a selective Na$^+$ pore blocker). It should be noted, however, that at the 300-ms time point late current block might not have reached steady state, particularly at the lowest concentrations. However, longer test pulses can activate variable levels of endogenous currents in the oocyte that interfere with accurate measurement of the very small, late Na$^+$ currents. As shown in Fig. 1E, late current block was concentration dependent such that an 89 µM dose was required to reach 50% in all three of the LQT3 mutant channels. Thus the concentration dependence of lidocaine block of late currents, although intermediate in effectiveness between tonic and phasic block, more closely resembled inactivated-state block. Given the uncertainty about whether late current blockade was measured in steady state, it seems possible that all of the block was associated with the inactivated state, but we cannot rule out a contribution from drug interaction with the resting or open states. Nonetheless, our results clearly show that when measured under identical conditions, lidocaine did not discriminate between the different LQT3 mutants. Moreover, additional observations described below suggest that late current block occurred primarily from interaction of the drug with the inactivated state.

The identification of tonic and phasic block with resting and inactivated states, respectively, assumes that inactivation is absent at the holding potentials used to measure tonic block. We measured steady-state inactivation (Fig. 2) using a standard voltage protocol of a 25-ms test pulse to $-10$ mV preceded by a 500-ms conditioning pulse to varying potentials from a holding potential of $-100$ mV. Peak test pulse currents were normalized to the maximum amplitude recorded under control conditions and plotted against the conditioning potential. The block by lidocaine in both WT and ΔKPQ channels was characterized by a concentration-dependent shift of the steady-state inactivation curves toward more negative potentials and by a distortion of the shape of the curve. Channel maximum availability saturated at $-100$ mV in the absence of drug, whereas in the presence of high concentrations of drug the curve did not reach plateau in the range $-100$ to $-150$ mV. This effect may account for the increased amount of tonic block observed at high lidocaine concentrations (Fig. 1) and suggests that block of late current may also be sensitive to the effects of drug on steady-state inactivation. Therefore, we tested whether depolarizing holding potentials could enhance the effectiveness...
of late current block. Typical effects of varying the holding potential on late current block in ΔKPQ at 50 µM lidocaine are shown in Fig. 3, A–C. Very negative holding potentials (Fig. 3, A and B) remove steady-state inactivation and appear to have little effect on late current block, but as the holding potential approached the midpoint of the steady-state inactivation curve (Fig. 3C), we observed a significant increase in late current block. A similar pattern is present in pooled data from all three LQT3 channels, but the increased block at a holding potential of −80 mV was only statistically significant for the ΔKPQ mutant. These results indicate that late current block is sensitive to the inactivated state of the channel and that the ΔKPQ mutant channel is at least as sensitive to lidocaine block as the N/S and R/H mutants.

Lidocaine blockade in single channels. In native Na^+ channels, lidocaine block produces a very stable inactivated, drug-bound state from which recovery is much slower than recovery from inactivation in the absence of drug (19). Therefore, the effectiveness of lidocaine in blocking late currents in N/S and R/H mutants was not surprising, because the gating defect (dispersed reopenings) appears to reduce the stability of the inactivated state at depolarized test potentials. In these channels, lidocaine is likely to increase the stability of the inactivated state and thereby restore normal gating through a reduction in the frequency of the dispersed reopenings. The observation that late currents generated by ΔKPQ had a similar sensitivity to lidocaine was more difficult to rationalize, because roughly one-half of the late current is contributed by burst activity, a gating mode in which the channels fail to inactivate (6). Channels in this mode might be resistant to lidocaine block, because they are in the open state most of the time. If such were the case, resistance to block associated with ΔKPQ burst openings should increase the relative contribution of the burst over dispersed openings to the ΔKPQ late current in the presence of lidocaine. An alternative explanation is that the open state in the ΔKPQ bursting mode might have an unusually high sensitivity to block (compared with normal channels or noninactivating mutants; Ref. 5) such that burst mode activity and dispersed reopenings are equally sensitive. A second alternative is that lidocaine stabilizes the inactivated state, and, as a result, the channels seldom enter the burst mode. We addressed these questions by single-channel analysis of ΔKPQ.

To determine whether lidocaine blocked channel openings during the burst mode, we measured dwell time in the open state for single-channel events during test pulses to 0 mV from a holding potential of −100 mV. Under cell-attached conditions, the midpoint potential of steady-state inactivation was typically shifted from −71 mV observed in whole cell recording to approximately −100 mV (similar observations have been reported for WT channels; Ref. 17). We therefore used 500-ms prepulses to −140 mV to fully remove inactivation. Figure 4 shows representative recordings in controls and after application of 10 µM lidocaine. In both sets of traces we observed burst activity, related to the slow gating mode in ΔKPQ channels, and short dispersed openings characteristic of all three mutant channels. The last pair of traces shows the ensemble average currents as described below. We analyzed open time and amplitude histograms of idealized records to obtain estimates of mean open time and unitary amplitude (Table 1). After application of 10 µM lidocaine, we observed fewer bursts and dispersed openings and more null traces (no detectable activity). However, as shown in Table 1, drug application had no effect on either the long open times associated with bursting or the short open times associated with dispersed reopenings. Moreover, unitary amplitudes were unaffected by
drug application. These results indicate that lidocaine does not act by blocking the open state in LQT3 channels.

We next determined whether lidocaine differentially blocked dispersed versus burst activity by estimating the contribution of bursting to the total ensemble average current. Bursts were defined as groups of openings with open times $\geq 6$ ms, based on our previous results (13), and traces containing at least one group of openings $\geq 6$ ms were averaged. A closed-time histogram was constructed from this collection of traces and fit to a biexponential distribution. We observed that $90\%$ of the distribution area was described by a time constant of $0.2$ ms and the remaining events by a time constant of $11$ ms. The shortest time constant was taken as the closed interval between events within a burst, and a burst was defined as a sequence of three or more openings separated by intervals $\geq 6$ ms. A dispersed opening was therefore a single event separated from its neighbors by closed intervals $\geq 6$ ms. To quantify the relative contributions of the bursts and dispersed openings, we separated the traces containing bursts and calculated an ensemble current from these selected traces. The burst ensemble current was then averaged to the total number of traces and normalized to the ensemble average current from the entire set of recordings. In the experiment illustrated in Fig. 4, the current from burst openings accounted for $67\%$ of the ensemble average current in control and $69\%$ when lidocaine was applied. In three experiments with $10$ µM lidocaine, the contribution of bursts to the ensemble average current was $62 \pm 9\%$ in control and $63 \pm 15\%$ after application of the drug. There was no significant difference between the two conditions compared by Student’s $t$-test or ANOVA. The difference in the contributions between control and $50$ µM lidocaine-treated cells were $2$ and $4\%$ in two other patches, respectively.

Table 1. Effect of lidocaine on single-channel parameters of $\Delta$KPQ late openings

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0 µM (n = 8)</th>
<th>10 µM (n = 3)</th>
<th>50 µM (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude, pA</td>
<td>$1.13 \pm 0.10$</td>
<td>$1.04 \pm 0.12$</td>
<td>$1.11 \pm 0.15$</td>
</tr>
<tr>
<td>$t_D$, ms</td>
<td>$0.41 \pm 0.02$ (61%)</td>
<td>$0.41 \pm 0.03$ (67%)</td>
<td>$0.43 \pm 0.06$ (69%)</td>
</tr>
<tr>
<td>$t_B$, ms</td>
<td>$4.62 \pm 0.19$ (39%)</td>
<td>$4.80 \pm 0.22$ (33%)</td>
<td>$4.76 \pm 0.26$ (31%)</td>
</tr>
</tbody>
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Averaged single-channel amplitudes and mean open times for dispersed ($t_D$) and burst ($t_B$) open events during late activity of $\Delta$KPQ in control and after application of extracellular lidocaine. All recordings were obtained in cell-attached patches during a 150-ms test pulse to 0 mV from a holding potential of $-100$ mV. Only last 130 ms of each trace were analyzed. Results pooled from set of 10 patches (some patches provided data in >1 test condition) with 600–900 traces/patch under each condition.

Fig. 4. Lidocaine blockade in single $\Delta$KPQ channels. Traces 1–9 show patch-clamp records from a cell-attached patch containing at least 5 channels. Currents were evoked by 0-mV, 150-ms test pulses from HP of $-100$ mV at a frequency of 0.2 pulses/s. Each test pulse was preceded by a $-140$-mV, 500-ms prepulse to remove resting inactivation. Dashed line indicates baseline (0 pA), and openings are negative events (downward deflections). Currents were filtered on-line at 3 kHz and digitized at 10 kHz. Traces were analyzed without additional filtering but are illustrated with 2-kHz filtering. Initial 20 ms of each record are blanked; only last 130 ms of each trace (late currents) were analyzed. In control (left) clusters of traces containing long burst openings were separated by more quiescent periods showing short dispersed openings. Bath application of $10$ µM lidocaine (right) reduced overall activity and prolonged interval between bursts and between dispersed openings. Bottom panels show total and burst components of ensemble average currents. Ensemble of traces containing bursting activity accounted for 67 and 69% of total in control and treated cells, respectively. Contribution of current from bursts to total ensemble average current was calculated from ratio of averaged current value during last 40 ms (AVG, dashed lines) for each condition. Nearly identical contribution of bursting activity to ensemble average current shows that lidocaine reduced late current in a nonselective fashion between bursting and nonbursting traces. Same result was obtained by analyzing means in 4 additional patches (total $n = 5$).
Because lidocaine does not act by blocking the open state in LQT3 channels, we asked whether late current block was associated with the inactivated state, an indication of which would be an increase in closed time between events. As shown in Fig. 5, A and B, the frequency of occurrence of both bursts and dispersed openings was reduced during application of lidocaine. Activity diaries were constructed before (Fig. 5A) and after (Fig. 5B) drug application to quantify the frequency of the bursts. In a very active control ΔKPQ patch (Fig. 5A), 276 traces showed burst activity, indicated by large spikes in the open probability diary, and 47 traces showed no activity (null trace) during the last 120 ms of the test pulse (after peak current has subsided) in a series of 705 steps to 0 mV (150 ms). After application of 50 µM lidocaine (Fig. 5B), we observed 51 bursts in a series of 485 active traces. Lidocaine reduced the occurrence of bursts from 39 to 10% and increased the fraction of null traces from 6 to 19% (Fig. 5C). The number of channels in the patch was estimated from the ratio of maximum peak transient (∼50 pA in this patch) to single channel (∼1 pA, Table 1) to be roughly 50 channels. In previous experiments (13), the late current accounted for 0.15 and 1.6% of the WT and ΔKPQ peak current, respectively. In a patch containing 50 channels, this translates to a contribution of no more than two channels during the late activity (unitary current ∼1 pA). Recordings of late current with overlaps of more than two current amplitude levels were discarded, and single transitions between closed and open states were easily discerned. To quantify the effect of lidocaine on the closed-time distribution between the short openings, we selected traces showing only dispersed activity and constructed a closed-time histogram (Fig. 5, D and E) in the same patch before and after drug application. In the control data of the patch analyzed in Fig. 5, the closed-time distribution was fitted by the sum of two exponentials with fast (τ₁) and slow (τ₂) time constants of 2 ms (61%) and 18 ms (39%), respectively. After lidocaine treatment (Fig. 5E), longer...
closed-time intervals were more frequent, as indicated by the rightward shift of the peak of the fit, and values of 2 ms (26%) and 29.6 ms (74%) for \( \tau_1 \) and \( \tau_2 \), respectively, were obtained. In a total of five patches, we observed shifts of the closed-time distribution toward the longer component and/or an increase in one or both of the time constants. Because the number of channels varied in each patch, we could not pool closed-time data from different patches or ascribe the drug-induced changes to a particular inactivation scheme. However, our qualitative comparison of the closed-time distribution for the dispersed reopenings is consistent with the channels spending more time in closed (drug bound, inactivated) states after lidocaine application.

Figure 5C shows the effect of lidocaine on the occurrence of bursting and null traces in the unsorted data set from the experiment described above. We observed average reductions of 14 ± 3% (n = 4) in the frequency of the bursts in patches treated with 10 µM and 17 ± 2% (n = 3) in patches treated with 50 µM lidocaine. The average increase in null traces was 3.5 ± 2% (n = 4) and 10.5 ± 0.4% (n = 3) in 10 µM and 50 µM lidocaine, respectively. We therefore observed more frequent and longer closed-time intervals between dispersed openings, a reduction in the frequency of the bursts, and an increase in the number of null traces after application of lidocaine consistent with the hypothesis of lidocaine-induced prolongation of dwell time in the inactivated state.

These results indicated that the drug did not exert an activity-specific blockade. Moreover, the reduction in the activity was not caused by changes in the mean open time during the bursts but rather by a prolongation of the intervals between open events of each type, consistent with the well-known action of lidocaine to slow recovery from the inactivated state (see, e.g., Ref. 4). This explanation presupposes that initiation of both dispersed and burst mode openings is inhibited when the channels are in the drug-bound, inactivated state. In the case of the dispersed reopenings, we postulate that the channels have already entered the inactivated state and that lidocaine inhibits reopening by slowing the recovery reaction. If a similar mechanism applied to burst mode activity, this would predict that the initiation of bursting would be inhibited by inactivation even in the absence of drug. Such an effect also would be consistent with our observation of holding potential dependence of late current blockade (Fig. 3) in \( \Delta \text{KPQ} \) whole-cell measurements.

We designed a series of experiments to explore this idea by testing whether inactivating prepulses affect the amount of burst activity recorded from \( \Delta \text{KPQ} \) channels in the absence of lidocaine. Under standard experimental conditions (see, e.g., Fig. 5), diaries of burst activity were obtained by repetitive test pulses preceded by a 500-ms hyperpolarizing prepulse (−140 mV) that removed resting inactivation. With each test pulse the channels start in the resting state and progress to open and inactivated states. Entry into the burst mode could occur from any or all of these states.

Figure 6 shows the results of varying the conditioning prepulse voltage such that before the test pulse was applied the channels were either completely resting (with inactivation removed by a prepulse to −140 mV) or partially inactivated (prepulse −70 mV). The peak ensemble average current measured at −10 mV was reduced by 85% after a depolarizing prepulse to −70
mV (Fig. 6, A–C). A diary of the late activity (Fig. 6D) showed that bursts were frequently observed during test pulses preceded by a −140-mV prepulse but strongly suppressed after switching to a prepulse to −70 mV. This experiment also suggests that the frequency of the dispersed reopenings was unaffected by the conditioning prepulse. In Fig. 6E, the cumulative integrals of the current traces are plotted against the trace number. In this plot, clusters of low-open probability product (NP_o, where N is no. of channels and P_o is mean open probability) traces (Fig. 6D), in which reopenings are the only form of activity, translate into line segments with a constant, shallow slope that occur throughout the experiment, whereas high-NP_o traces containing bursts result in brief intervals of steep slope that were less frequent during application of −70 mV prepulses.

The reduction in bursting varied significantly from one patch to another and with the number of channels active under the patch. In two other experiments (not shown), both bursts and peak current were completely abolished by −70-mV prepulses without significant changes in the shallow slopes in the integral plot. The fluctuations in the availability of the channels correlated with the change in bursting activity in these experiments, but overall, inactivating prepulses reduced bursting by 64 ± 11% (n = 6).

DISCUSSION

We previously reported that mexiletine (a lidocaine analog) was more effective in blocking the late component of Na^+ current than the peak current (13), and a similar observation has been made for lidocaine block in ΔKPQ channels expressed in mammalian cells (2). These studies, however, did not address questions about the mechanism of the late current block. Previous reports (6, 13) showed that the ΔKPQ mutation facilitated the entry of the channels into a mode of gating characterized by bursts of long openings, whereas in the N/S and R/H mutations (13) persistent current was generated by short, dispersed reopenings. On the basis of the low affinity of lidocaine for block of the open state (4, 5), we expected to see less effective block in ΔKPQ channels, but our data did not support this hypothesis. The main evidence comes from the equal contribution of the bursting activity to the late current with or without lidocaine. We observed the same reduction of the open probability for both types of activities through a prolongation of the closed times between the events.

It has been proposed that the bursts are caused by the switching of channels into a slow mode of gating (6, 25), in which the entry and the recovery from inactivation are altered, and that the dispersed activity is caused by the destabilization of the absorbing inactivated state, allowing return to the open state. Thus the two types of activity involve different gating transitions of the channel. Our single-channel data, however, showed that depolarizing prepulses that inactivated ΔKPQ channels selectively inhibited the burst openings over the dispersed activity. This selective reduction indicated that channels residing in the resting states are more likely to enter a slow mode of gating than inactivated channels, but channels that become inactivated during sustained depolarization are likely to reopen in a dispersed fashion. This result clearly shows that the initiation of bursting is unlikely to occur in channels that are inactivated, and this observation helps explain why bursts are suppressed by lidocaine, a drug that preferentially binds to and stabilizes channels in the inactivated state. This property of the burst mode in ΔKPQ channels distinguishes it from previously identified modal gating in native cardiac Na^+ channels, in which switching from burst mode to dispersed reopenings was found to occur independently of prepulse potentials (7). Therefore, although the ability to switch into a bursting mode appears to be an intrinsic property of Na^+ channels, the regulatory mechanism that controls mode switching may be different in normal and mutant channels.

Our results also showed that the late current block was dependent on the holding potential, and part of the block was produced before the test pulse was applied. At the normal resting potential (approximately −80 mV), the LQT3 channels are partly inactivated and the block of the inactivated channels by lidocaine will decrease the probability of slow gating. Therefore, the block of late current by lidocaine reflects binding to the inactivated state and will be increased compared with tonic block. During the test pulse to 0 mV, the ΔKPQ channels fully activate within 2 ms and are maximally inactivated within 20 ms. Lidocaine block, therefore, increased after the channels inactivated, i.e., after the initial 20 ms of the pulse, and an extra block develops after the peak current has resolved. In unclamped cells, during the plateau phase of the action potential, more channels would be inactivated and trapped in an inactivated, blocked state from which reopenings would be less likely to occur (stabilizing effect).

Recently, however, Wang et al. (33) suggested that mexiletine inhibits late activity through an open-state blocking mechanism. They base their conclusions on the observation that the onset of mexiletine block of inactivated channels is too slow to account for late current block and therefore must be caused by open channel block. No single-channel data are presented, and this conclusion conflicts with previous studies of mexiletine’s mechanism of action in native Na^+ channels. For instance, Ono et al. (24) showed that mexiletine block of activated channels proceeds at a much slower rate than block of inactivated channels and, under physiological conditions, has no effect on single-channel open time (23). Moreover, although a numeric simulation in which open channel blockade can effectively suppress persistent current originating from bursts of long openings (33), the same model cannot account for the observation that the drugs are equally effective against N/S and R/H channels that only exhibit dispersed reopenings. When the drug block rates used by Wang et al. (33) are applied in model channels that exhibit either dispersed reopenings (13) or bursting (6), we find that at 10 µM mexiletine would be
predicted to reduce the persistent currents by 27 and 75%, respectively. Moreover, because of the short open times of the dispersed reopenings, the onset of block would be at least three times slower. Our experimental results clearly indicate that open channel block is not essential for suppressing late current in LQT3 mutants. Moreover, from a clinical standpoint, drugs that have a strong open channel blocking potency (i.e., class 1A blockers such as quinidine and disopyramide, unlike class 1B drugs such as lidocaine; Ref. 18) may, in fact, pose a risk of further prolonging the action potential by blocking K+ currents (8) and therefore would be inappropriate treatments for long Q-T syndrome.

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