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Common molecular determinants of local anesthetic, antiarrhythmic, and anticonvulsant block of voltage-gated Na^+ channels

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ABSTRACT **Voltage-gated Na^+ channels are the molecular targets of local anesthetics, class I antiarrhythmic drugs, and some anticonvulsants. These chemically diverse drugs inhibit Na^+ channels with complex voltage- and frequency-dependent properties that reflect preferential drug binding to open and inactivated channel states. The site-directed mutations F1764A and Y1771A in transmembrane segment IVS6 of type IIA Na^+ channel α subunits dramatically reduce the affinity of inactivated channels for the local anesthetic etidocaine. In this study, we show that these mutations also greatly reduce the sensitivity of Na^+ channels to state-dependent block by the class Ib antiarrhythmic drug lidocaine and the anticonvulsant phenytoin and, to a lesser extent, reduce the sensitivity to block by the class Ia and Ic antiarrhythmic drugs quinidine and flecainide. For lidocaine and phenytoin, which bind preferentially to inactivated Na^+ channels, the mutation F1764A reduced the affinity for binding to the inactivated state 24.5-fold and 8.3-fold, respectively, while Y1771A had smaller effects. For quinidine and flecainide, which bind preferentially to the open Na^+ channels, the mutations F1764A and Y1771A reduced the affinity for binding to the open state 2- to 3-fold. Thus, F1764 and Y1771 are common molecular determinants of state-dependent binding of diverse drugs including lidocaine, phenytoin, flecainide, and quinidine, suggesting that these drugs interact with a common receptor site. However, the different magnitude of the effects of these mutations on binding of the individual drugs indicates that they interact in an overlapping, but nonidentical, manner with a common receptor site. These results further define the contributions of F1764 and Y1771 to a complex drug receptor site in the pore of Na^+ channels.**

Voltage-gated Na^+ channels are responsible for the initiation and propagation of action potentials in both nerve and muscle cells (1, 2). The main structural component of Na^+ channels is the 260-kDa α subunit, which forms the Na^+ selective pore and other structures necessary for channel function. In mammalian Na^+ channels, the α subunit associates with one or two smaller auxiliary subunits designated β1 and β2. Na^+ channel function is regulated by voltage-dependent transitions among three sets of functionally distinct conformational states. At hyperpolarized membrane potentials, most Na^+ channels are in closed resting states. In response to membrane depolarization, channels rapidly convert to an open state that conducts Na^+ ions and then to a nonconducting, inactivated state. The opening and subsequent inactivation of Na^+ channels results in a transient inward current that inactivates within a few milliseconds.

Class I antiarrhythmic drugs such as lidocaine, quinidine, and flecainide (3, 4) as well as some anticonvulsants including phenytoin and carbamazepine (5) act by inhibiting ionic currents through voltage-gated Na^+ channels. Tertiary amine local anesthetics, like procaine and etidocaine, which are chemically related to lidocaine, act in a similar manner (6). The efficacy of these drugs stems from their ability to selectively inhibit Na^+ channels during abnormal membrane depolarizations and rapid bursts of action potentials that characterize cardiac and neuronal pathologies (7–9). The selectivity of local anesthetic, antiarrhythmic and anticonvulsant drugs for depolarized Na^+ channels results from the preferential binding of these drugs to the open and inactivated channel states that predominate at depolarized membrane potentials rather than the resting channel states that predominate at more hyperpolarized membrane potentials. This state-dependent drug action can be explained by an allosteric model in which a modulated drug receptor is in a low affinity conformation when the channel is resting, and converts to a high affinity conformation when the channel is open or inactivated (8, 9).

In a previous study (10), we used alanine-scanning mutagenesis to investigate the role of residues in the IVS6 transmembrane segment of the rat brain type IIA Na^+ channel α subunit in state-dependent block by the local anesthetic etidocaine. We found that mutations F1764A and Y1771A in segment IVS6 reduced the affinity of inactivated channel states for etidocaine by up to two orders of magnitude. In contrast, the mutations had much less effect on the affinity of resting channels for this drug. We proposed that the native residues at these positions form part of the receptor for etidocaine and are especially important in the inactivated receptor conformations. Here we show that these residues are also important for the actions of the local anesthetic/class I antiarrhythmic lidocaine and the anticonvulsant phenytoin, whereas they are less important for the local anesthetics quinidine and flecainide. Our results suggest that these drugs may block at overlapping receptor sites on the channel protein and may be stabilized in their receptor site by interactions with both common and divergent amino acid residues.

EXPERIMENTAL PROCEDURES

Construction and Expression of Mutants. A detailed description of the procedures for site-directed mutagenesis, *Xenopus* oocyte isolation, and mRNA injection is given in ref. 11.

Electrophysiological Recording. Oocytes were examined 2–6 days after injection by two-electrode voltage clamp recording using a Dagan CA-1 voltage clamp (Dagan Instruments, Minneapolis). Pulses were applied and data were acquired by using a personal computer-based data acquisition system (Basic-Fastlab; Indec Systems, Sunnyvale, CA). During recording, the bath was continuously superfused with frog Ringer solution (115 mM NaCl/2.5 mM KCl/1.8 mM $CaCl_2$/10 mM Hepes, pH 7.2). Drugs were applied in the superfusate. Drug solutions were made from 100 mM (for

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Abbreviation: WT, wild type.

lidocaine, quinidine, and flecainide) or 20 mM (for phenytoin) stock solutions in dimethyl sulfoxide. Lidocaine, quinidine, and phenytoin were obtained from Sigma. Flecainide was a generous gift from 3M Pharmaceuticals.

RESULTS AND DISCUSSION

Mutations F1764A and Y1771A Reduce Tonic and Frequency-Dependent Block by Antiarrhythmic and Anticonvulsant Drugs. Local anesthetic, antiarrhythmic, and anticonvulsant Na^+ channel blockers inhibit Na^+ currents in both a tonic and frequency-dependent manner. These two components of block are illustrated in Fig. 1*A*, which shows macroscopic Na^+ currents recorded from a *Xenopus* oocyte expressing wild type (WT) Na^+ channels. The dashed trace shows current evoked by a stimulus pulse to 0 mV from a holding potential of −80 mV in control conditions. The membrane potential was then returned to −90 mV and 200 μM lidocaine was washed into the bath. The solid traces show currents elicited by a 10-Hz pulse train 10 min after beginning lidocaine exposure. Current evoked by the first pulse of the train was reduced in comparison to control. This inhibition, referred to as tonic block, developed at −90 mV in the absence of channel activation. Since most channels were in resting states at this potential, the block was mainly due to drug binding to resting channels. The weak tonic inhibition reflects the low affinity of this conformational state for lidocaine. During subsequent pulses in the train, an additional component of block developed. This depolarization- or frequency-dependent component of block was due to the transient availability of higher affinity open and/or inactivated channels during each stimulus pulse. After rapid pulsing was terminated, the level of block returned to its original tonic level within a few seconds as the channels converted to low affinity resting states upon repolarization. Frequency-dependent block of WT channels was just detectable when pulses were applied at 2 Hz, and increased with stimulus frequency, up to at least 20 Hz (Fig. 1*D*).

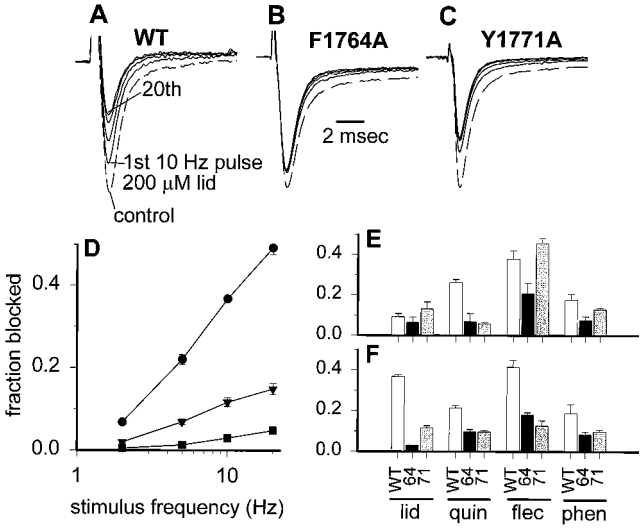

FIG. 1. Tonic and frequency-dependent block of WT and mutant Na^+ channels by lidocaine, quinidine, flecainide, and phenytoin. (*A–C*) Lidocaine inhibition of Na^+ currents elicited in oocytes expressing WT (*A*), F1764A (*B*), or Y1771A (*C*) channels. Currents were evoked by 15-msec-long depolarizations to 0 mV from a holding potential of −90 mV. The dashed traces show currents elicited in control. The solid traces were obtained 10 min after changing the bath perfusate to one containing 200 μM lidocaine. Currents evoked by 1st, 2nd, 5th and 20th pulses of 10-Hz pulse trains are shown. Current traces have been normalized so that control current magnitude is approximately equal for WT and each mutant. (*D*) Frequency-dependence of lidocaine block of WT (●), F1764A (■) and Y1771A (▼) channels. Trains of 20 stimulus pulses were applied at various frequencies in the presence of 200 μM lidocaine. The graph shows the (peak current during the 20th pulse)/(peak current during the 1st pulse) of the train plotted as a function of stimulus frequency. In this and subsequent figures, the data points show means ± SEMs, unless otherwise indicated. (*E* and *F*) Tonic (*E*) and frequency-dependent block (*F*) of WT and mutant channels by lidocaine (200 μM; 10 Hz pulses), quinidine (500 μM; 1 Hz), flecainide (200 μM; 1 Hz), and phenytoin (200 μM; 20 Hz). (*E*) Tonic block is plotted as the fraction of the control current that is blocked by drug. (*F*) Frequency-dependent block is the additional fraction of current that is blocked by a pulse train as described in *D*.

Frequency-dependent block of mutants F1764A and Y1771A by lidocaine was dramatically reduced (Fig. 1 *B–D*, *F*), but there was no significant effect on tonic block (Fig. 1 *B*, *C*, and *E*). The reduction in frequency-dependent block was seen over a broad range of stimulus frequencies (Fig. 1*D*). Since frequency-dependent block reflects drug binding to open and inactivated channels, these results suggest that mutating either residue F1764 or Y1771 to alanine caused a strong reduction in the affinity of one or both of these states. We have previously reported a similar finding for the structurally related local anesthetic, etidocaine (10). Apparently residues F1764 and Y1771 are important molecular determinants of action of these tertiary amine local anesthetic and antiarrhythmic drugs on open and/or inactivated Na^+ channels.

Lidocaine and etidocaine have similar chemical structures, so it is not surprising that they share molecular determinants on the Na^+ channel protein. However, other clinically important local anesthetic, antiarrhythmic, and anticonvulsant drugs with diverse chemical structures also act as tonic and frequency-dependent inhibitors of Na^+ channels. Thus, they too might interact with residues F1764 and Y1771. To test this, we examined the action of three additional Na^+ channel blocking drugs: the class Ia antiarrhythmic quinidine, the class Ic antiarrhythmic flecainide, and the anticonvulsant phenytoin. Fig. 1 *E* and *F* summarize the degree of tonic and frequency-dependent block of WT, F1764A, and Y1771A channels by lidocaine, quinidine, flecainide, and phenytoin. Pulse frequencies (see legend to Fig. 1) were chosen to give substantial, but not complete, frequency-dependent block of WT by each drug. Frequency-dependent block was significantly reduced in the mutant channels compared with WT for each drug (Fig. 1*F*). Mutation F1764A also significantly reduced tonic block by quinidine, flecainide, and phenytoin compared with control, and Y1771A reduced tonic block by quinidine (Fig. 1*E*), suggesting that these two residues also play a role in the action of these agents on resting channels. Thus, F1764 and Y1771 are important for frequency-dependent block and, to a lesser extent, for tonic block by these drugs. The disruption of frequency-dependent block by these mutations is substantially stronger for lidocaine than for quinidine, flecainide, or phenytoin. The remaining experiments will describe the role of residues F1764 and Y1771 in state-dependent block by these drugs in more detail.

Lidocaine Binds Preferentially to Inactivated Channels, Whereas Quinidine and Flecainide Bind Preferentially to Open Channels. Cumulative frequency-dependent block results from enhanced binding of drugs to channels during each depolarizing pulse and failure of the drug to unbind completely between pulses. Since frequency-dependent block was reduced in mutant channels, it was important to determine how each drug interacted with open and inactivated channels during depolarizations. Lidocaine, quinidine, and flecainide are strong frequency-dependent blockers at high stimulus frequencies, whereas phenytoin is a weaker frequency-dependent blocker. Thus, this question was addressed differently for the different drugs.

To investigate frequency-dependent block by lidocaine, quinidine, and flecainide, Na^+ currents were elicited, in the presence of drug, by depolarizing pulses to 0 mV before

(control) and after (test) a 10-Hz train of twenty 50-msec pulses to varying conditioning potentials (Fig. 2*A*). The extent of frequency-dependent block that developed during the conditioning train was measured as the ratio of peak currents elicited by the test and control pulses (test/control). The voltage range over which frequency-dependent block developed for each drug was compared with the voltage ranges over which activation and inactivation took place in order to correlate drug binding with channel state.

With lidocaine, current amplitude was strongly reduced after a train of conditioning pulses to −50 mV (Fig. 2*B*). At this conditioning potential, a large fraction of channels inactivated during each conditioning pulse, but there was no detectable Na^+ current through activated channels. Thus, lidocaine block at −50 mV reflected drug binding to channels during depolarizations, but channel opening was not required. When block was examined over a range of potentials (Fig. 2*D*), the dependence of block on conditioning pulse potential closely followed the voltage dependence of steady-state channel inactivation (dashed line), as expected for selective drug binding to inactivated states. At more depolarized potentials, where the probability of channel opening was high, drug binding to open channels may also have contributed to block. However, it was not possible to determine the additional contribution of lidocaine binding to open channels with this protocol. Preferential binding to inactivated channels was also an important mechanism of phenytoin block as shown in the next section.

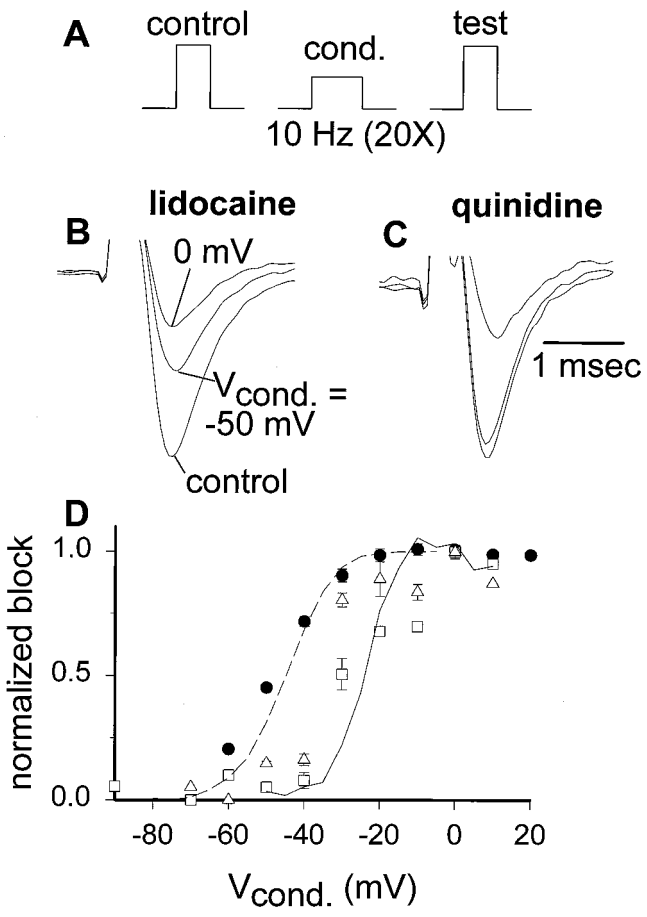

FIG. 2. Voltage dependence of frequency-dependent block of WT channels by lidocaine, quinidine, and flecainide. (*A*) The stimulus protocol used to determine the voltage dependence of frequency-dependent block. A depolarization to 0 mV (control) was followed by a 10-Hz train of twenty 50-msec pulses to a variable conditioning potential (cond.). The effect of the train was assessed by a second pulse to 0 mV (test). Drug concentrations were the same as in Fig. 1 (*B* and *C*). Current traces from experiments with lidocaine (*B*) and quinidine (*C*) elicited by a control depolarization before and by test depolarizations after conditioning trains to −50 mV or 0 mV. (*D*) The voltage dependence of frequency-dependent block for lidocaine (●), quinidine (□), and flecainide (△). Block was determined by the ratio of test/control currents, scaled so that the maximum block for each drug equaled 1.0, and plotted as a function of conditioning pulse potential. The dashed line is the mean of steady-state inactivation curves with maximal inactivation equaling 1, determined using 100-msec prepulses before drug application for each oocyte used in these experiments. The solid line is the mean integral of conductance at each potential $[\int I/(V - V_{rev})]$ where $\int I$ is the integral of the currents evoked by test pulses to each potential before drug application, V is the test pulse potential, and V_{rev} is the reversal potential. The integral conductance was normalized for comparison to the drug-block data.

For quinidine and flecainide, there was virtually no frequency-dependent block after conditioning pulses to −50 mV. However, strong block developed with depolarizations to more positive potentials, where channel activation was significant (Fig. 2 *C* and *D*). The voltage dependence of block by quinidine was shifted positively in comparison to steady-state inactivation, but was approximately described by the integral of the whole cell conductance at each test potential (solid line). This integral gives a measure of the total time that channels are open at each potential. The voltage dependence of block by flecainide was also shifted to more positive voltages in comparison with steady-state inactivation, but was not shifted to as positive voltages as with quinidine. These results are consistent with previous findings and suggest that frequency-dependent block by quinidine and flecainide requires channel opening (12–14).

Mutations F1764A and Y1771A Reduce the Affinity of Inactivated Channels for Lidocaine and Phenytoin. Since lidocaine (Fig. 2) and phenytoin (see below) bound to depolarized channels without requiring them to open, whereas quinidine and flecainide required channel opening, different protocols were used to assess effects of mutations on block by the two types of drugs. In this section, we address effects of mutations on lidocaine and phenytoin block of resting and depolarized channels.

Resting and inactivated channels are in dynamic equilibrium, with hyperpolarized membrane potentials favoring the resting states, and depolarized potentials favoring the inactivated states. Only resting channels are available to open during depolarizations. Thus, the proportion of available Na^+ channels as a function membrane potential can be determined by assessing the amplitudes of Na^+ currents elicited by stimulus pulses applied from varying holding potentials. For WT channels, inactivation was observed for holding potentials more positive than -70 mV, and the midpoint of the holding potential versus availability relationship was −53 mV (Fig. 3*A*). After application of lidocaine, the midpoint of the relationship was shifted 12 mV negative (Fig. 3*A*). This negative shift in channel availability ($\Delta V_{1/2}$) can be explained by preferential drug binding to inactivated channels compared with resting channels, causing a shift in the equilibrium between these two sets of functional states favoring inactivation (8, 9, 15). The magnitude of $\Delta V_{1/2}$ increased with increasing lidocaine concentration up to at least 2 mM (Fig. 3*C*). Phenytoin also shifted $V_{1/2}$ to more negative potentials in a concentration-dependent manner (Fig. 3*D*). The F1764A and Y1771A mutations substantially reduced $\Delta V_{1/2}$ for both lidocaine and phenytoin over the entire range of drug concentrations (Fig. 3 *B–D*), indicating that these mutations reduced preferential drug binding to inactivated states. In contrast, quinidine and flecainide did not shift availability curves for WT or mutant channels (not shown), as expected for drugs that require channels to open in order to bind.

To quantify effects of F1764A and Y1771A on lidocaine and phenytoin binding to inactivated Na^+ channels more accurately, we determined concentration–effect relationships by

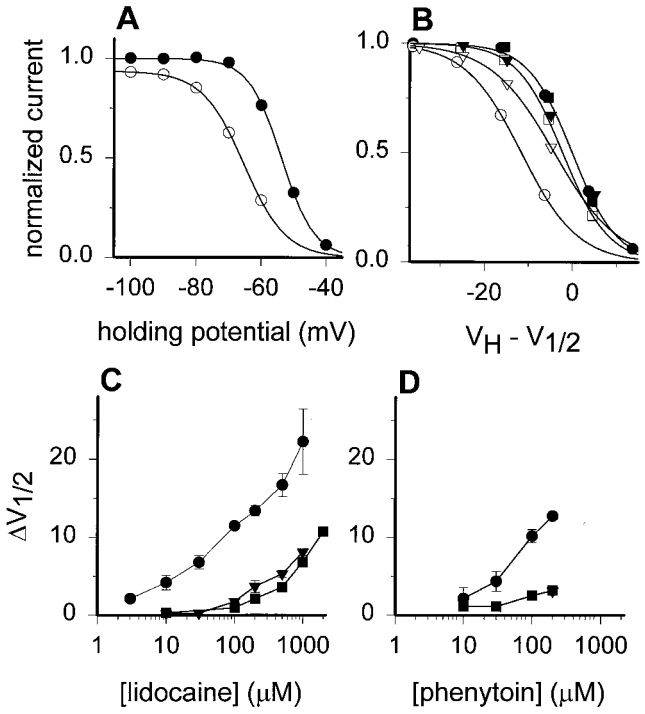

FIG. 3. Lidocaine- and phenytoin-dependent shifts in the voltage dependence of channel availability for WT and mutant channels. (*A*) A typical availability curve obtained from an oocyte expressing WT channels in control (●) and after application of 200 μM lidocaine (○). The graph shows the amplitude of Na^+ currents elicited by stimulus pulses to 0 mV, given 10 sec after stepping to the indicated holding potentials. Both sets of data points were normalized with respect to the largest currents obtained in control conditions from hyperpolarized holding potentials. The smooth lines are according to $1/\{1 + \exp[(V_{hold} - V_{1/2})/s]\}$ where V_{hold} is the holding potential, $V_{1/2}$ is the midpoint of the curve, and s is a slope factor. (*B*) Typical availability curves for WT (●, ○), F1764A (■, □), and Y1771A (▼, ▽) in control (solid symbols) and with 200 μM lidocaine (open symbols). The WT experiment is the same as in *A*. To better demonstrate the shifts in the voltage dependence of availability with drug application, the current amplitudes for both control and drug curves were normalized to 1.0 and the data were shifted along the voltage axis so that the control curves in each experiment superimposed with $V_{1/2} = 0$ mV. (*C* and *D*) The drug-dependent shift in channel availability ($\Delta V_{1/2}$) was determined from $V_{1/2,drug} - V_{1/2,control}$ using $V_{1/2}$ values obtained from fits of the data as described in *A*. The graphs show mean $\Delta V_{1/2}$ as a function of lidocaine (*C*) or phenytoin (*D*) concentration for WT (●), F1764A (■), and Y1771A (▼).

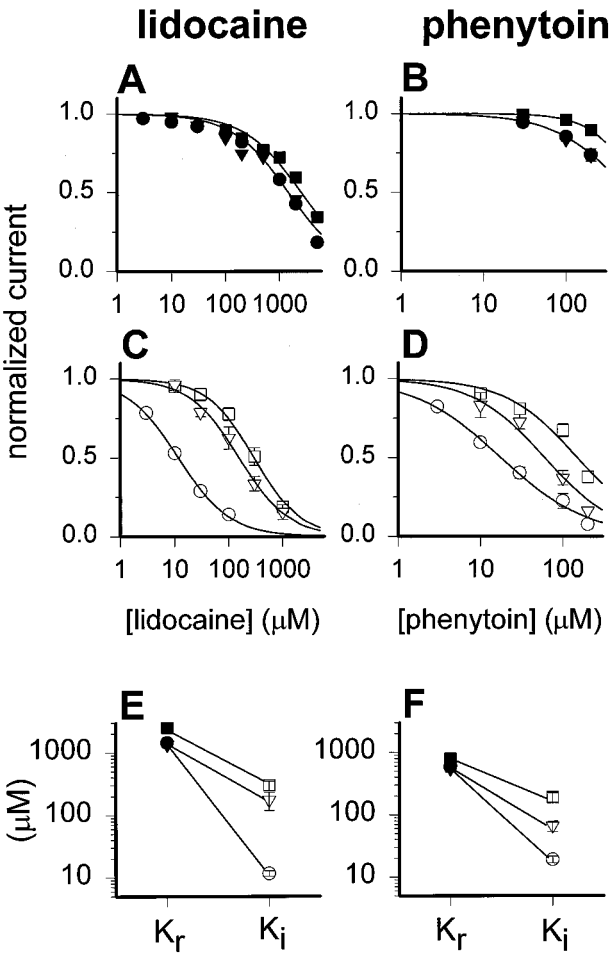

FIG. 4. Affinity of resting and inactivated states of WT and mutant channels for lidocaine and phenytoin. (*A* and *B*) Mean current normalized with respect to control in oocytes expressing WT (●), F1764A (■), or Y1771A (▼) channels, elicited by stimulus pulses to 0 mV, given 15 sec after stepping the holding potential to −120 mV, as a function of lidocaine (*A*) or phenytoin (*B*) concentration. The smooth lines were fit to $1/(1 + [drug]/K_r)^n$, where K_r is the midpoint of the curve and n is the Hill coefficient. Due to the low solubility of phenytoin, it was not possible to use concentrations higher than 200 μM. Thus, the midpoints for the phenytoin curves, obtained by extrapolating fits from the available data points, are approximate. (*C* and *D*) Mean, normalized amplitudes of currents in oocytes expressing WT (○), F1764A (□), or Y1771A (▽) channels, evoked by pulses applied 15 sec after stepping the holding potential to −40 mV (WT and Y1771A) or −35 mV (F1764A), as a function of lidocaine (*C*) or phenytoin (*D*) concentration. A more positive holding potential was used for F1764A because this mutation shifted the voltage dependence of inactivation approximately 5 mV positive compared with WT. The smooth lines are according to $1/(1 + [drug]/K_i)^n$. (*E* and *F*) Mean K_r (filled symbols) and K_i (open symbols) for lidocaine (*E*) and phenytoin (*F*), obtained from oocytes expressing WT (●, ○), F1764A (■, □), or Y1771A (▼, ▽) channels.

using experimental protocols that isolated either resting or inactivated states. To assess resting affinity, we examined the concentration dependence of block of Na^+ currents evoked by depolarizing stimulus pulses applied from a holding potential of −120 mV (Fig. 4 *A* and *B*). At this holding potential, virtually all channels were in resting states. Thus, these dose-effect relationships gave estimates of K_r, the equilibrium binding constant for drug binding to resting channels (15). For WT channels, K_r was 1448 ± 138 μM for lidocaine and 583 ± 148 μM for phenytoin, values that are similar to estimates of affinities of these drugs for resting native Na^+ channels (16, 17). For Y1771A, K_r for both drugs was virtually identical to WT. For F1764A, K_r was shifted to ≈1.5-fold higher concentrations. Although these changes were statistically significant, neither mutation caused a dramatic decrease in the affinity of resting channels for lidocaine or phenytoin.

Because inactivated channels do not open in response to depolarization, drug binding to inactivated states must be assessed indirectly by its effect on the availability of resting channels. In a cyclic four-state modulated-receptor model, in which a channel can be in resting, inactivated, resting-blocked, or inactivated-blocked states (15), the midpoint of concentration-effect curves for inhibition of Na^+ current approaches K_i, the equilibrium constant for block of inactivated states, at holding potentials where channel inactivation is near 100% (15, 16, 18). Therefore, we estimated K_i from concentration-effect relationships at depolarized holding potentials (−40 mV or −35 mV), where channel inactivation was greater than 95%, using oocytes injected with high concentrations of mRNA to obtain measurable Na^+ currents with these experimental conditions.

For WT channels, K_i measured in this way was 11 ± 2.7 μM for lidocaine and 19 ± 4.2 μM for phenytoin (Fig. 4 *C* and *D*).

Thus, inactivated WT channels displayed 120-fold and 32-fold higher affinities for lidocaine and phenytoin, respectively, than resting channels. These values are similar to those obtained for binding of lidocaine (15, 17) and phenytoin (16) to inactivated native Na^+ channels. Both mutations F1764A and Y1771A reduced affinity of inactivated channels compared with WT (Fig. 4 *C–F*). This difference was especially striking for lidocaine, where K_i was increased 24.5-fold for F1764A and 12.9-fold for Y1771A (Fig. 4 *C* and *E*). For phenytoin, K_i was increased 8.3-fold for F1764A and 3.3-fold for Y1771A (Fig. 4 *D* and *F*). Thus, for both drugs, mutations F1764A and Y1771A caused strong reductions in inactivated state affinity compared with WT, but had little or no affect on resting state affinity. Evidently, these two residues are important determinants of the affinity of drug binding to the inactivated conformation of the receptor site.

Mutations F1764A and Y1771A Reduce Affinity of Resting and Open Channels for Quinidine and Flecainide. In addition to requiring open channels in order to bind, quinidine and flecainide also differed strikingly from lidocaine or phenytoin in the rate at which frequency-dependent block was reversed when rapid pulsing was terminated. Block by lidocaine and phenytoin returned to the tonic-block level within seconds when pulsing was terminated. In contrast, frequency-dependent block by quinidine or flecainide required many minutes to recover (not shown), much as it does for block by charged local anesthetics like QX314 (10). Apparently, the dissociation of these drugs from closed channels was extremely slow, as if the drugs were trapped within the channels when they closed. One possible explanation for these observations is that the channel activation and/or inactivation gates act as physical barriers, preventing drug access to and escape from the receptor site (19, 20). Because drug binding and unbinding during rapid pulses took place at an appreciable rate only when channels were open, frequency-dependent block during a pulse train can be regarded as the gradual equilibration of drug binding to the open channel state (21, 22). Thus, the concentration dependence of the steady-state level of frequency-dependent block by these drugs gives an estimate of K_o, the dissociation constant for drug binding to the open state of the channel.

At each drug concentration tested, flecainide caused a measurable level of tonic block in the first test pulse of a pulse train and an increased level of block at steady state in the 20th pulse in a pulse train (Fig. 5*A*). The dependence of tonic block of resting WT channels, K_r, and state-dependent block of open WT channels, K_o, on flecainide concentration is illustrated in Fig. 5*B*. For WT channels, open-state affinity was 2.5-fold higher than resting-state affinity for flecainide (Fig. 5 *B* and *C*). This contrasts with a 120-fold differential between inactivated and resting channels for lidocaine. For mutant Y1771A, K_r for flecainide was similar to WT and K_o was increased 1.5-fold. For mutant F1764A, K_r and K_o for flecainide were increased 2.0- and 2.5-fold, respectively (Fig. 5*C*). Effects of a similar magnitude were observed for quinidine (Fig. 5*D*). Thus, although the mutations F1764A amd Y1771A significantly reduced both resting and open channel affinity for these drugs, the effects were weak in comparison to the effects of these mutations on the inactivated-state affinity for lidocaine and phenytoin.

A Common Receptor Site for Local Anesthetic, Antiarrhythmic, and Anticonvulsant Na^+ Channel Blockers. The most striking finding of this study is that the chemical and physical properties of the residues at positions 1764 and 1771 in the IVS6 transmembrane segment of the type IIA Na^+ channel are critical determinants of the efficacy of two clinically important Na^+ channel blockers, the class Ib antiarrhythmic lidocaine and the anticonvulsant phenytoin. Mutation of the native residues at either position to alanine strongly disrupts the binding of these drugs to inactivated states, but has much less

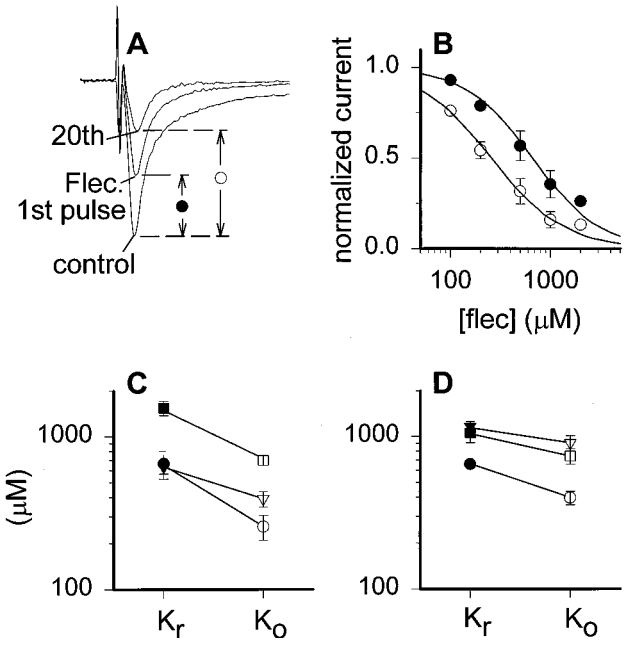

FIG. 5. Affinity of resting and open channel states of WT and mutant channels for quinidine and flecainide. (*A*) Current traces from a typical experiment with flecainide on WT channels. The stimulus protocol was the same as that of Fig. 1*A*. Resting channel block was the amount of block of the channel during the first pulse of a 2-Hz pulse train from a holding potential of −120 mV (●), just as for lidocaine and phenytoin (Fig. 4 *A* and *B*). Open channel block was measured as the steady-state amount of block during the 20th pulse in the train (○). (*B*) The concentration dependence of resting channel block (●) and open channel block (○) for inhibition of WT channels by flecainide. The smooth lines are according to $1/\{1 + [[\text{drug}]/(K_r \text{ or } K_o)]^n\}$ where K_o is the affinity constant for block of open channels (*C* and *D*). Mean K_r (filled symbols) and K_o (open symbols) of WT (●, ○), F1764A (■, □), and Y1771A (▼, ▽) for flecainide (*C*) and quinidine (*D*).

effect on block of resting channels. We have previously shown that the F1764A and Y1771A mutations also strongly disrupt the action of the local anesthetic etidocaine on inactivated Na^+ channels (10). Thus, residues F1764 and Y1771 appear to be important for the action of chemically diverse drugs that selectively act on inactivated Na^+ channels. These two residues line up on the same face of the putative IVS6 α helix (10), and we have proposed that they face toward the inner lumen of the channel pore, the likely site for drug binding. They may become accessible to the drugs as a result of the conformational changes associated with channel inactivation, and stabilize drug binding by interactions with charged, hydrophobic, and/or aromatic moieties on the drug molecules (23–25). Mutation of the native residues to alanine would then be expected to destabilize drug binding by reducing hydrophobicity and/or aromaticity at these sites.

The F1764A amd Y1771A mutations had similar, but weaker, effects on the binding of the Class Ia and Ic antiarrhythmics quinidine and flecainide which bind preferentially to open Na^+ channels. The quaternary lidocaine derivative, QX314, also binds preferentially to the open Na^+ channel, but its binding is strongly disrupted in mutant F1764A (10). This suggests that the comparatively weak effects of mutations F1764A and Y1771A on binding of flecainide and quinidine are not caused solely by their preferential binding to open Na^+ channels but rather are due to their different structures that dictate stronger interactions with other amino acid residues in their receptor site. Thus, residues F1764 and Y1771 may be very important for stabilizing the binding of lidocaine, QX314, etidocaine, and phenytoin to open and inactivated Na^+ chan-

nels, but less important for binding of quinidine and flecainide in all channel conformations.

Our results support the hypothesis that part of the local anesthetic/antiarrhythmic/anticonvulsant receptor of the voltage-gated Na^+ channel is formed by amino acid residues at positions 1764 and 1771 in the IVS6 transmembrane segment of the α subunit. Consistent with this model, the effects of these mutations on drug block are not correlated with effects on channel activation or inactivation (10). However, it is also possible that the mutated residues are not directly involved in drug binding but specifically destabilize channel conformations that are critical for binding specific classes of drugs but not for activation and inactivation of the Na^+ channel. These amino acid residues are conserved between neuronal and cardiac Na^+ channels and so are common determinants of drug action in nerve and heart. In addition, it is likely that residues in other regions of the channel also contribute to the drug receptor site and that these residues may interact preferentially with drugs such as flecainide and quinidine. The unique biophysical and pharmacological properties of these drugs may depend on such differential molecular interactions. Since biophysical evidence suggests that local anesthetics and related drugs bind within a cytoplasmic vestibule of the channel pore (8, 26, 27), likely regions for additional determinants of drug binding include the S6 transmembrane segments of the other three homologous domains of the channel, which may line the walls of the vestibule, and the SS1–SS2 regions in each domain, which form the selectivity filter of the pore, and thus, may define the roof of the vestibule (1). In addition, the channel inactivation gate, formed by the intracellular loop connecting homologous domains III and IV of the channel α subunit (for review, see ref. 1), may also be important for drug binding and action (28). Further experiments to completely map the drug receptor site will give insights into the molecular basis of state-dependent channel block and the different blocking properties of various Na^+ channel blockers. This molecular characterization may facilitate the rational design of more effective therapeutic agents.

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1. Catterall, W. A. (1992) *Physiol. Rev.* **72** (Suppl.), S15–S48.
2. Hille, B. (1992) *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA).
3. Vaughn Williams, E. M. (1984) *J. Clin. Pharmacol.* **24,** 129–147.
4. Harrison, D. C. (1985) *Am. J. Cardiol.* **56,** 185–187.
5. Rogawski, M. A. & Porter, R. J. (1990) *Pharmacol. Rev.* **42,** 223–286.
6. Butterworth, J. F. & Strichartz, G. R. (1990) *Anesthesiology* **72,** 711–734.
7. Catterall, W. A. (1987) *Trends Pharmacol. Sci.* **8,** 57–65.
8. Hille, B. (1977) *J. Gen. Physiol.* **69,** 497–515.
9. Hondeghem, L. M. & Katzung, B. G. (1977) *Biochim. Biophys. Acta* **427,** 373–398.
10. Ragsdale, D. S., McPhee, J. C., Scheuer, T. & Catterall, W. A. (1994) *Science* **265,** 1724–1728.
11. McPhee, J. C., Ragsdale, D. S., Scheuer, T. & Catterall, W. A. (1995) *J. Biol. Chem.* **270,** 12025–12034.
12. Zilberter, Y. I., Starmer, C. F. & Grant, A. O. (1994) *Am. J. Physiol.* **266,** H2007–H2017.
13. Snyders, D. J. & Hondeghem, L. M. (1990) *Circ. Res.* **66,** 565–579.
14. Anno, T. & Hondeghem, L. M. (1990) *Circ. Res.* **66,** 789–803.
15. Bean, B. P., Cohen, C. J. & Tsien, R. W. (1983) *J. Gen. Physiol.* **81,** 613–642.
16. Kuo, C.-C. & Bean, B. P. (1994) *Mol. Pharmacol.* **46,** 716–725.
17. Nuss, H. B., Tomaselli, G. F. & Marban, E. (1995) *J. Gen. Physiol.* **106,** 1193–1209.
18. Bean, B. P. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6388–6392.
19. Strichartz, G. R. (1973) *J. Gen. Physiol.* **62,** 37–57.
20. Yeh, J. Z. & Tanguy, J. (1985) *Biophys. J.* **47,** 685–694.
21. Courtney, K. R., Kendig, J. J. & Cohen, E. N. (1978) *J. Pharmacol. Exp. Ther.* **207,** 594–604.
22. Chernoff, D. M. (1990) *Biophys. J.* **58,** 53–68.
23. Bokesh, P. M., Post, C. & Strichartz, G. R. (1986) *J. Pharmacol. Exp. Ther.* **237,** 773.
24. Sheldon, R. S., Hill, R. J., Taouis, M. & Wilson, L. M. (1991) *Mol. Pharmacol.* **39,** 609–614.
25. Zamponi, G. W. & French, R. J. (1993) *Biophys. J.* **65,** 2335–2347.
26. Cahalan, M. D. (1978) *Biophys. J.* **23,** 285–311.
27. Cahalan, M. D. & Almers, W. (1979) *Biophys. J.* **27,** 39–56.
28. Bennett, P. B., Valenzuela, C., Chen, L. Q. & Kallen, R. G. (1995) *Circ. Res.* **77,** 584–592.