Interactions of Flecainide With Guinea Pig Cardiac Sodium Channels

Importance of Activation Unblocking to the Voltage Dependence of Recovery

Takafumi Anno and Luc M. Hondeghem

Effects of flecainide, a potent antiarrhythmic agent, on sodium channel availability was investigated in guinea pig single cardiac cells by the whole-cell voltage-clamp technique. Sodium current (I_{Na}) experiments were performed at 17°C, and maximum upstroke velocity (V_{max}) experiments were performed at 37°C. Flecainide (3 μM) caused little tonic block, but reduced sodium channel availability in a use-dependent manner. The latter effect was accentuated by depolarization and attenuated by hyperpolarization. Long (200-msec) and short (10-msec) depolarizations yielded similar use-dependent block. These results indicate that flecainide has a low affinity for rested (R) and inactivated (I) channels but a high affinity for activated ones (A). In each of these states, the channels can bind to drug to form the corresponding RD, ID, and AD states. Recovery from flecainide block consisted of two components. An initial fast component was strongly voltage dependent: with increasing hyperpolarization, recovery developed more quickly and to a larger extent. At 17°C, the mean time constant shortened from 132±81.6 msec at −120 mV to 46.9±34.1 msec at ±160 mV (kinetics were too fast for accurate measurement at 37°C). A later slow component was largely voltage independent: at 37°C, the mean time constant was 9.8±3.2 seconds at −100 mV and 10.7±3.8 seconds at −75 mV. The slow component of recovery was similarly independent of voltage at 17°C. In terms of the modulated-receptor theory, our results indicate that the fast recovery depends on availability for unblocking (RD) but occurs during activation (AD⇒A). Indeed, when the RD state is maximized by strong hyperpolarization, activation unblock was also maximized. However, during depolarization to −100 mV, availability for activation unblock declined with a time constant of 98±12 msec (RD⇒ID). Therefore, the voltage-dependent fast unblocking is mostly due to priming of the RD state (RD⇐ID), and the voltage-independent slow unblock reflects dissociation of flecainide from closed states (RD⇒R and ID⇒I). We conclude that flecainide interacts with sodium channels preferentially in the activated state, whereas unblocking occurs via two separate pathways: activated and closed states. Furthermore, drug association with channels shifts the voltage dependence of closed-state transitions (RD⇒ID) and their kinetics toward more negative potentials. (Circulation Research 1990;66:789–803)

Flecainide is a potent antiarrhythmic agent that blocks cardiac sodium channels in a time- and voltage-dependent fashion: reduction of maximum upstroke velocity (V_{max}) of the action potential is frequency dependent, diastolic recovery from block occurs slowly (15–20 seconds), and the steady-state relation between V_{max} and membrane potential is shifted toward more negative potentials.1–3 A detailed study of the state-dependent actions of this drug using voltage-clamp techniques has not been done. Moreover, because the actions of the drugs are strongly temperature dependent, we executed sodium current (I_{Na}) experiments at 17°C and V_{max} experiments at 37°C.4–8

According to Hodgkin and Huxley,9 the sodium channel can exist in three primary states: rested (R), activated (A), and inactivated (I). When these states are drug associated, they are termed RD, AD, and ID, respectively. The transitions between these states

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From the Department of Medicine and Pharmacology, Stahlman Cardiovascular Research Program, Vanderbilt University, Nashville, Tennessee.

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Address for correspondence: Luc M. Hondeghem, MD, PhD, Department of Medicine and Pharmacology, Stahlman Cardiovascular Research Program, Medical Center North, CC2209, Vanderbilt University, Nashville, TN 37232.

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are governed by membrane potential. Antiarrhythmic agents interact with these three states with characteristic association (k) and dissociation (l) rate constants.\textsuperscript{10,11} In terms of the modulated receptor theory,\textsuperscript{10,11} clinically useful antiarrhythmic agents usually have a low affinity for the rested state, while the affinity for activated or inactivated states is much greater.\textsuperscript{12} It is not known how flecainide interacts with the primary states of the cardiac sodium channel.

It is well established that diastolic recovery from block is voltage dependent.\textsuperscript{12,13} Recovery from block is usually hastened by hyperpolarization; it becomes slower on depolarization. This voltage dependence of recovery from block has been attributed to a faster dissociation rate constant for rested channels than for inactivated channels (I_\text{R} > I_\text{I}).\textsuperscript{11} However, recovery from block is the sum of closed-state unblocking (RD⇒R and ID⇒I) and activation unblocking (AD⇒A).\textsuperscript{8,14–19} According to Snyder and Hondeghem,\textsuperscript{19} activation unblocking critically depends on availability of drug-associated channels (RD). The availability for unblocking of drug-associated channels is augmented by hyperpolarization until inactivation is completely removed (all ID⇒RD). Recovery from inactivation in drug-associated channels occurs at more negative potentials than in drug-free channels, and the slope of steady-state availability curve for the drug-associated channels appears reduced.\textsuperscript{19} However, the kinetics for the state transitions of quinidine-associated channels were too fast to allow accurate determination of their voltage dependence. Such studies are inherently difficult at the level of V_{max} or I_{Na} measurements, because transition between states in drug-associated channels may be invisible since drug-associated channels may not conduct sodium. If voltage-dependent kinetics of drug-associated channels can be determined indirectly, such information is very important to the understanding of drug action. Indeed, it has been proposed that drug binding does not modify the voltage dependence of channel gating.\textsuperscript{20}

It has been established that quaternary compounds have a slow recovery from use-dependent block.\textsuperscript{21} Moreover, there is considerable evidence that closed-channel unblocking is directly linked to the neutral species of the drug.\textsuperscript{22,23} If only neutral drugs can readily diffuse from the receptor site while the channel is closed,\textsuperscript{10} then one would not a priori expect closed-state unblocking to be voltage dependent. Stated more strongly, the possibility exists that voltage dependence of recovery from block results primarily from activated-state unblocking; that is, the time constant of RD⇒R and ID⇒I may not be very different or voltage dependent. This hypothesis can easily be tested by studying recovery from block at various potentials, while using a test pulse that keeps activation unblocking minimal or at least relatively constant.

In the present study we therefore addressed the following questions: 1) What are the primary states for block of sodium channels by flecainide? 2) How are these interactions modified by temperature? 3) Is diastolic recovery from block voltage dependent? 4) Does activation unblocking (AD⇒A) exist, and how is it modulated by voltage? 5) Does closed-state unblocking exist (RD⇒R or ID⇒I), and is it modulated by voltage? 6) Is the voltage dependence of recovery from block primarily due to activation unblock (AD⇒A)? 7) Do inactivation and recovery from inactivation in drug-associated channels (ID⇒RD) have time and voltage dependence that is different from that in drug-free channels (I⇌R)?

### Materials and Methods

#### Preparations and Solutions

Experimental data were derived from 78 single ventricular cells obtained from adult guinea pig hearts. Cells were dispersed by a procedure previously described in detail.\textsuperscript{8,19,24} Briefly, guinea pig hearts were perfused using a Langendorff apparatus. After washing out the blood, the heart was perfused for 3 minutes with a nominally free calcium solution and for approximately 10 minutes with 0.05%collagenase (types II and III, Worthington, Freehold, New Jersey) and 0.02%trypsin (GIBCO Laboratories, Grand Island, New York) solution. The heart was then minced, and the cells were dissociated by gentle trituration. After several washes, the cells were kept in modified KB medium\textsuperscript{25} for 2 hours and then stored in Medium 119 (GIBCO Laboratories) at 37°C in an environment of 95% O_2–5% CO_2 for 2–10 hours until use. Only cells that were rod-shaped and remained quiescent with clear cross-striations were selected for the study.

The bath had a volume of 0.5 ml and was perfused at 1 ml/min with a buffered salt solution that was heated to 37±0.5°C (for V_{max} experiments) or cooled to 17±0.5°C (for I_{Na} experiments) by means of a Peltier device (Midland Ross, Cambridge, Massachusetts). For the V_{max} experiments, the internal solution consisted of (mM) KCl 110, MgCl\textsubscript{2} 2, CaCl\textsubscript{2} 1, NaCl 8, EGTA 11, HEPES 10, and K\textsubscript{2}ATP 5 (pH 7.2). The external solution consisted of (mM) NaCl 145, KCl 4, CaCl\textsubscript{2} 1.8, MgCl\textsubscript{2} 1, glucose 10, and HEPES 10 (pH 7.35). For the I_{Na} experiments the intracellular solution contained (mM) NaF 10, CsF 110, CsCl 20, MgCl\textsubscript{2} 2, EGTA 2, and HEPES 10 (pH 7.2). The extracellular solution was composed as follows (mM): NaCl 20, CsCl 110, CaCl\textsubscript{2} 2, CoCl\textsubscript{2} 3, tetraethylammonium chloride 10, glucose 10, and HEPES 10 (pH 7.35).

Flecainide (flecainide acetate, 3M, Riker Laboratories, Minneapolis, Minnesota) was dissolved in water (850 μM) and added to the perfusion solution. The concentration of flecainide was 3 μM unless otherwise stated.
Electrophysiological Techniques

Single cells were voltage-clamped by the whole-cell recording mode of the patch-clamp technique by use of an Axoclamp-2 (Axon Instruments, Burlingame, California) in the single-electrode discontinuous (V<sub>max</sub>) and continuous (I<sub>Na</sub>) modes. To optimize voltage control, we selected small cells (less than 100×20 μm) and made electrodes with a resistance ranging from 2 to 4 MΩ for V<sub>max</sub> experiments and from 0.4 to 1 MΩ for I<sub>Na</sub> experiments. Cell capacitance and series resistance were compensated by analog circuits. Command potentials were generated by a 12-bit D/A converter (Tecmar, Cleveland, Ohio) controlled by custom software.

At 37°C, V<sub>max</sub> was used as an indicator of sodium channel availability because above 20°C it is difficult to adequately control the sodium current. The membrane potential was clamped by a switching (6–10 kHz) single-electrode voltage clamp. To elicit a free-running upstroke, the clamp was switched to the current-clamp mode for 10 msec (see arrows in top panel of Figure 1). During this time, a square current pulse (2 msec) was applied, and its amplitude was adjusted so as to induce an upstroke with constant latency. However, in contrast to multicellular preparations, latency appeared to have relatively little effect on V<sub>max</sub>. The upstroke of the action potential was differentiated by use of a circuit that was linear up to 1,000 V/sec, and its peak was captured with a peak detector. After the 2-msec stimulus pulse, the cell was clamped to zero current to obtain a free-running upstroke. The membrane voltage (top two tracings of Figure 1, top) and their V<sub>max</sub> (bottom two tracings of Figure 1, top) were sampled at 40 kHz and analyzed on a pulse-by-pulse basis. The stimulus intensity was adjusted until all measured upstrokes in any train had a constant latency (±0.5 msec), and the potential at V<sub>max</sub> was constant (±3 mV). Under physiological circumstances (37°C, K<sub>c</sub>=4 mM, K<sub>i</sub>=120 mM), the cells had a resting potential of −81±3.6 mV (n=14) and an action potential shape that closely resembled that of multicellular preparations (left side of Figure 1, top). V<sub>max</sub> was larger in single-cell preparations (300–500 V/sec) than in multicellular preparations probably because the membrane action potential of single cells experiences less electrical loading than the propagated action potential of multicellular preparations.

When measuring I<sub>Na</sub> at 17°C, only cells that generated less than 10 nA maximum peak sodium current, that were free of oscillation, and that provided a current-voltage relation with a descending limb extending over at least 25 mV were retained. The adequacy of the voltage clamp under these circumstances has been previously demonstrated. Currents were filtered with an 8-pole analog Bessel filter at 5 kHz (∼3 dB) and recorded digitally at a sampling frequency of 20–100 kHz. A standard test pulse to −10 mV for 10 msec was used to record I<sub>Na</sub>.

![Figure 1. Top panel: Methods for voltage clamping and measurement of the maximum upstroke velocity (V<sub>max</sub>). The left side of top panel shows the recording of an action potential. The right side of top panel shows that the membrane potential is voltage-clamped (VC) except during the upstroke, where it was current-clamped (CC). The upstroke was elicited by a brief 2-msec current pulse followed by a free-running upstroke (zero current clamp). The upstroke was differentiated, and its peak was captured by a peak-detecting circuit and is shown at the bottom on the right. The tracings in control and in the presence of flecainide (3 μM) are marked by C and F, respectively. Those tracings were recorded at a cycle length (CL) of 500 msec (37°C), and latency was adjusted to within ±0.5 msec. Note the greatly expanded time scale during the upstroke of the action potential. Bottom panel: Voltage dependence of use-dependent block. Results obtained at 37°C using V<sub>max</sub> are shown at various holding potentials: −90 and −65 mV under control conditions (open symbols) and −105 to −65 mV in the presence of 3 μM flecainide (filled symbols). Test pulse is shown in inset as a free-running upstroke (dashed line) followed by a 10-msec clamp pulse to +20 mV. Note that every other pulse was recorded. After an unblocking pulse train and a 20-second rest period at the holding potential, the membrane was clamped for 1 second to the indicated potential after which the pulse train was applied.](image-url)
out the drug and to make repeat measurements. In the few experiments where this was attempted, the washout was incomplete.

**Induction of Block and Unblock**

In the present experiments, we studied development of block as well as the process of unblocking. The former was best measured after maximum relief of block; the latter was best studied after induction of maximal block. Since relief of block and development of block in the presence of flecainide proceeded so slowly when holding at a fixed potential, we used the procedure described by Snyders and Hondeghem. To maximize block a fast train of brief pulses was applied from a less negative holding potential, which was adjusted so as to minimize normal inactivation and to induce block as quickly as possible. In contrast, to elicit unblocking a train of pulses from very negative potentials was applied, to which we will refer as the unblocking train. For \( V_{\text{max}} \) experiments, a train consisting of 200 brief pulses at a cycle length of 50 msec from around \(-80 \) mV was used to induce maximum block; to elicit unblock 20 pulses from the holding potential of \(-120 \) mV were applied at a cycle length of 200 msec. For \( I_{\text{Na}} \) experiments 50–200 depolarizations (10 msec) to \(+20 \) mV at a cycle length of 100 msec from a holding potential near \(-110 \) mV were used as a block-induction train. This provided nearly complete block in the presence of flecainide. For the unblocking train 10-msec pulses to \(-10 \) mV at a cycle length of 500 msec were applied from a holding potential of \(-180 \) mV.

**Data Analysis**

Data are represented as mean±1 SD unless stated otherwise. Analysis of variance (ANOVA) with Scheffé's test was used for critical difference among multiple means, and Student's \( t \) test was used for comparison between two means. Differences were considered significant at \( p<0.05 \). All fitting of exponential and Boltzmann curves to the data was accomplished with a nonlinear least-squares algorithm. Other smooth curves drawn through the data were obtained by using the cubic spline fit in a scientific graphing package (Biosoft, Milltown, New Jersey).

**Results**

Under control conditions at \( 37^\circ \)C, \( V_{\text{max}} \) became maximal when holding at potentials more negative than \(-70 \) to \(-80 \) mV; at \( 17^\circ \)C, \( I_{\text{Na}} \) reached its maximum value at potentials more negative than \(-100 \) mV (about 10 minutes after breaking in the cell) to \(-120 \) mV (usually 60 minutes later) or more negative. A similar hyperpolarizing shift of inactivation in the cardiac cell has been described by others. Therefore, we used \(-90 \) mV as the holding potential in the \( V_{\text{max}} \) experiments and \(-140 \) mV in the \( I_{\text{Na}} \) experiments, unless stated otherwise.

**Use-Dependent Block and Unblock**

**Use-dependent block.** Flecainide (3 \( \mu \)M; holding potential, \(-90 \) mV) minimally reduced \( V_{\text{max}} \) of a test pulse after a 20-second rest period (5.8±4.9% ; \( n=11 \), \( p<0.05 \) by paired \( t \) test), when \( I_{\text{Na}} \) (holding potential, \(-120 \) mV) was not reduced. However, on application of a train of depolarizations, the block by flecainide increased with each pulse until \( V_{\text{max}} \) and \( I_{\text{Na}} \) declined to a reduced steady-state value. This use-dependent reduction of \( V_{\text{max}} \) was strongly dependent on the holding potential (Figure 1, bottom). Under control conditions (open symbols), application of a pulse train at a cycle length of 500 msec resulted in little or no change of \( V_{\text{max}} \) over the potential range of \(-90 \) to \(-65 \) mV. In the presence of flecainide (filled symbols), use-dependent block resulted at all potentials shown, but it was markedly larger at the less negative holding potentials. The use-dependent reduction of \( V_{\text{max}} \) by 40 pulses was 13.7±4.8% \((n=4) \) at \(-90 \) mV but was 49.1±10.8% at \(-65 \) mV \((p<0.01; n=5) \). Similar results were obtained in \( I_{\text{Na}} \) experiments: under control conditions \( I_{\text{Na}} \) exhibited no use-dependent decline over the potential range of \(-140 \) to \(-100 \) mV, but in the presence of flecainide \( I_{\text{Na}} \) was reduced to 92.4±2.4% \((n=4) \) at \(-140 \) mV and to 51.4±8.7% \((n=4) \) at \(-100 \) mV \((p<0.01; n=4) \). In addition, use-dependent reduction of \( V_{\text{max}} \) and \( I_{\text{Na}} \) was also strongly dependent on cycle length (not shown), that is, more marked the shorter the cycle length.

The kinetics of block development were also markedly dependent on membrane potential. Indeed, for \( V_{\text{max}} \) at \(-90 \) mV (Figure 1, bottom) and \( I_{\text{Na}} \) at \(-110 \) mV (Figure 2, right), steady-state block was approached during a train of 60 pulses, whereas at more positive potentials use-dependent block did not reach steady state in 60 depolarizations. Use-dependent block at more negative potentials could be well approximated by a single exponential. However, at \( 37^\circ \)C, as the holding potential was made less negative, the sum of at least two exponentials was required to fit the onset of block; at \(-65 \) mV, a fast pulse constant of about 1.7 pulses and a slow pulse constant of about 100 pulses were required.

**Activation dependence of block.** We compared (by ANOVA) the amount of use-dependent block among three different pulse trains; short (10-msec) duration pulses with a 500-msec interpulse interval and long (200-msec) interpulse interval with a 500- or 300-msec interpulse interval. All pulses were from a holding potential of \(-90 \) mV to \(+20 \) mV. Under control conditions, application of all three 40 pulse trains resulted in little or no reduction of \( V_{\text{max}} \) (<5%). In the presence of flecainide (3 \( \mu \)M), both long and short pulses with a 500-msec interpulse interval resulted in a significant reduction of \( V_{\text{max}} \) (18.2±11.5% and 16.3±10.6% respectively, \( p<0.01; n=7 \)). However, the reductions of \( V_{\text{max}} \) by long and short pulses were not significantly different from each other. Moreover, application of a train of long pulses separated by
300-msec intervals induced 26.1±12.9% block (n=7), which was significantly larger than either of the former two trains with the 500-msec interpulse interval (P<0.05). Thus, number of activations per unit time and diastolic interval are more important than duration of the depolarizations.

Voltage dependence of block and unblock. In the presence of flecainide (3 μM), application of a pulse train when most of the channels were drug free resulted in development of use-dependent block (Figure 2, left). INa declined on a pulse-by-pulse basis from 7.8 nA to approach a steady state of 5.2 nA at the holding potential of −110 mV. When the identical pulse train was applied after a block induction train that reduced INa to less than 2 nA, INa increased to reach the same steady state obtained by use-dependent block.

The amplitude and kinetics of use-dependent blocking were also voltage dependent: unblock became faster and was more marked as the holding potential was made more negative. Application of 10 pulses at a cycle length of 500 msec (only for 5 seconds) resulted in unblock to 77% of control value at −140 mV but only 47% at −110 mV (Figure 2, right). Similar observations were obtained in three INa experiments by using test pulses with even shorter length (5 msec). Again results obtained in three experiments of Vmax were qualitatively similar.

Fast and Slow Components of Unblocking

Recovery from use-dependent block has long been known to be strongly voltage dependent.13 This voltage dependence has been interpreted as follows: recovery from block occurs more quickly in rested than in inactivated channels.11,12 However, since unblocking associated with activation depends strongly on the holding potential,19 the question arises whether recovery in the absence of activations has a voltage dependence of its own.

In Figure 3 (top), the increase of Vmax after block induction as a function of time is shown at two holding potentials (−100 and −75 mV). Under control conditions the recovery from inactivation was virtually complete within 100 msec at both potentials. In the presence of flecainide (3 μM), recovery of Vmax was much slower. Moreover, from 100 msec to 30 seconds, the amplitude of Vmax at −100 mV was always larger than that at −75 mV (n=6, p<0.05 by paired t test). However, the time constants for recovery from use-dependent block observed at −100 and −75 mV were not significantly different (9.8±3.2 and 10.7±3.8 seconds, respectively; p>0.4; n=6). Thus, most of the difference in recovery between the two potentials must have developed early in the recovery process; later recovery developed with relatively similar kinetics at both potentials.

To characterize these two processes more precisely, we measured recovery of INa after induction of nearly complete block with 6 μM flecainide (Figure 3, bottom). Under control conditions, INa recovered mostly within 200 msec (<20 msec in Table 1). This recovery process reflecting removal of normal inactivation could be well-fitted by a single exponential. In the presence of drug, as the holding potential during the recovery period was clamped more negatively, more recovery developed. However, careful examina-
Figure 3. Graphs showing voltage dependence of recovery from block. Top panel: Late phase of recovery (0.1–30 seconds), which was recorded from a single experiment at 37°C using maximum upstroke velocity (Vmax) in the absence (open) and presence (filled) of 3 μM flecainide at −75 and −100 mV. τ, time constant. Bottom panel: Early (5 msec to 2 seconds) phase of recovery at 17°C using sodium current (INa) in the presence of 6 μM flecainide. In both instances, maximum block was first induced by a fast pulse train indicated by the filled box in the figure insets. The membrane potential was clamped to various potentials for various times, and a test pulse was then used to monitor recovery from block.

Potential revealed that recovery was composed of at least two components. Recovery started with a fast component that had an amplitude that increased and a time constant that shortened as the membrane potential was made more negative (Table 1). At all potentials more negative than −160 mV, the fast recovery process reached similar levels in 200 msec, but recovery was less complete at more positive potentials.

After the initial fast phase, recovery proceeded much more slowly and did not reach full unblocking in 1 minute. Although at 17°C the kinetics were too slow to be fully characterized, the rate of the slow process appeared to be similar at potentials ranging from −120 to −160 mV: the amplitudes of percent recovery between 200 msec and 50 seconds was 17.8% at −160 mV and 16.9% at −120 mV (Table 1).

To further isolate the slow phase of recovery at different potentials, the fast component was minimized by preceding the test pulse with a 2-second prepulse to −110 mV.17 Under these circumstances, the amplitude of recovery of INa, from 5 to 30 seconds was not different at potentials ranging from −180 to −120 mV (ANOVA, Table 2). Only at −100 mV did recovery appear to be diminished (p<0.05). However, we cannot be sure that the reduction at −100 mV could not be due to incomplete removal of inactivation resulting from a hyperpolarizing shift of the inactivation curve.30,31

Thus, both Vmax and INa results demonstrated that recovery from block is voltage dependent, but most of the voltage dependence is associated with the initial fast phase of recovery. The later slower recovery phase may not be dependent on membrane potential.

Relative contribution of activation unblock. To examine the contribution of use-dependent unblock to the recovery Vmax and INa, standard recovery from block and use-dependent unblock were compared in a single preparation. After induction of block (solid black box in inset of Figure 4, left) and after a variable recovery interval at variable potentials, a train of test pulses at 5 Hz was applied. In Vmax experiments, when holding at −100 mV (Figure 4, left panel), 10 test pulses induced much faster unblocking (open circles) than diastolic recovery without pulses (solid symbols). Indeed, Vmax increased from 202 to 310 V/sec during a train of pulses starting at 100 msec and ending at 1,900 msec. To obtain a similar level of Vmax without multiple activations, one had to wait until 10 seconds had elapsed, and some of this unblocking could have occurred during the test pulse. Thus, unblocking during activations occurs markedly faster than in their absence.

Furthermore, use-dependent unblock could be observed even as late as 10 seconds. At all times, use-dependent unblock could be satisfactorily approximated by a single-exponential process that approached a steady state of 328±10 V/sec with a beat constant of 5.2±1.4. Similar results were obtained in five other preparations.

In INa experiments (Figure 4, right panel), a 5-Hz train of 20 test pulses also produced marked unblocking. Activation unblocking (open symbols) was much faster than the standard recovery (filled symbols). In addition, activation unblocking was clearly voltage dependent. After 20 pulses (of 4 seconds), amplitudes of unblock were 28.4±11.0% at −120 mV, 60.9±11.2% at −140 mV, and 79.6±10.5% at −160 mV of control value (n=6), and beat constants were 7.0±1.5 at −120 mV (n=7), 6.2±1.1 at −140 mV (n=6), and 6.1±1.1 at −160 mV (n=5). Most importantly, the increase of INa by a pulse train at each potential was significantly larger than that of a single pulse in a standard recovery protocol (by paired t
TABLE 1. Voltage Dependence of Recovery From Inactivation and Rate of Unblock

<table>
<thead>
<tr>
<th>Amplitude of recovery (%)</th>
<th>−200 mV</th>
<th>−180 mV</th>
<th>−160 mV</th>
<th>−140 mV</th>
<th>−120 mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (200 msec)</td>
<td>93.3±9.0</td>
<td>87.5±9.1</td>
<td>94.2±7.7</td>
<td>94.8±7.7</td>
<td>92.5±14.1</td>
</tr>
<tr>
<td>Flecainide (200 msec)</td>
<td>26.0±4.1</td>
<td>24.1±3.7</td>
<td>24.8±2.1</td>
<td>19.4±2.9</td>
<td>9.8±4.4</td>
</tr>
<tr>
<td>Flecainide (50 seconds)</td>
<td>45.6±5.8</td>
<td>42.6±5.8</td>
<td>46.9±34.1</td>
<td>66.8±59.2</td>
<td>132.0±81.6</td>
</tr>
<tr>
<td>Time constant (msec)</td>
<td>&lt;5 (2)</td>
<td>&lt;5 (2)</td>
<td>8.5±4.6</td>
<td>15.0±3.7</td>
<td>19.6±5.4</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD when number of experiments (in parentheses) was three or greater. Amplitude of recovery represents the percentage of recovery from normal inactivation under control conditions and the percentage of recovery from use-dependent block by flecainide (6 μM). Time constant refers to a monoeXponential fit as a function of time within first 200 msec and correlation coefficients greater than 0.98.

*Significance of difference from 200-msec recovery with p<0.01 (unpaired t test).
†Significance of difference from control with p<0.01 (unpaired t test).
‡Significance of difference from control with p<0.05 (unpaired t test).

To characterize the use-dependent unblock per pulse for $V_{\text{max}}$ and $I_{\text{Na}}$ we plotted the increase per pulse as a function of normalized $I_{\text{Na}}$ for the preceding pulse. Such a plot gives a linear line of unblock. The slope of this line is an indicator of fractional unblock. Extrapolation of this line to 100% block yields the percent of channels that would unblock during one activation if all channels were blocked by drug (Table 3). Unblock per pulse for $V_{\text{max}}$ increased with hyperpolarization from less than 20% at −85 mV to 48% at −120 mV. Unblock per pulse for $I_{\text{Na}}$ was markedly smaller than for $V_{\text{max}}$ but it similarly increased with more negative holding potentials.

Voltage dependence of activation unblock. It was previously shown by Snyder and Hondeghem that the availability for activation unblocking of quinidine-associated channels ($h'$-curve in Reference 19) is shifted toward more negative potentials and has a shallower slope than that of the standard availability curve, that is, the inactivation curve ($h$-curve of Reference 19). To study the relation of activation-associated unblocking with holding potential, a 60-pulse train at a cycle length of 500 msec was applied at various potentials after induction of maximal block by flecainide (see insets of Figure 5). The availability curves were fitted with Boltzmann equations (Table 4). In control, $V_{\text{max}}$ was maximal at potentials more negative than −70 mV (Figure 5, left) but declined steeply on depolarization and became unmeasurably small at potentials more positive than −50 mV ($h$-curve). When determining this curve in the presence of flecainide but after an unblocking train, the curve was only slightly reduced and shifted to more negative potentials by a small amount (changes were not statistically significant).

In contrast, when plotting $V_{\text{max}}$ of the first pulse of the unblocking train as a function of potential (filled diamond), the availability curve for activation unblocking ($h'$-curve) was markedly reduced and shifted to more negative potentials (−16.2±2.8 mV; p<0.001 by ANOVA). At the same time, the slope factor of the Boltzmann fit was significantly increased by +4.1±1.2 mV (p<0.001 by ANOVA). Although unblocking occurred during the pulse train, the increase of $V_{\text{max}}$ was rather small at potentials more positive than −70 mV. Availability then increased as the membrane potential was made more negative, until a maximum was reached near −100 mV. Thus, after block induction by flecainide, most of the increase in $V_{\text{max}}$ occurred over the potential range where $V_{\text{max}}$ did not increase in control (−70 to −100 mV).

Qualitatively similar changes were observed at 17°C for $I_{\text{Na}}$ (Figure 5, right). Changes of the standard availability curve were not statistically different in the presence and absence of flecainide. In contrast, availability for activation unblocking after induction of block ($h'$-curve) was shifted substantially in the negative direction (−33.9±3.8 mV; p<0.001 by ANOVA) and had a slope factor that was increased by +6.0±1.5 mV (p<0.001 by ANOVA). It should be noted that the observed voltage shift of availability curves was significantly (p<0.001) larger at 17°C than at 37°C.

TABLE 2. Voltage Dependence of Percent Slow Unblock in Flecainide

<table>
<thead>
<tr>
<th>Recovery time</th>
<th>−180 mV</th>
<th>−160 mV</th>
<th>−140 mV</th>
<th>−120 mV</th>
<th>−100 mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 seconds</td>
<td>16.5±4.2</td>
<td>16.8±4.7</td>
<td>15.5±5.3</td>
<td>12.6±6.5</td>
<td>9.7±3.8</td>
</tr>
<tr>
<td>30 seconds</td>
<td>29.6±7.6</td>
<td>28.6±13.3</td>
<td>29.2±8.1</td>
<td>22.6±7.2</td>
<td>14.8±5.5</td>
</tr>
<tr>
<td>Difference (30−5 seconds)</td>
<td>13.1±7.9</td>
<td>11.6±5.5</td>
<td>13.7±4.6</td>
<td>10.0±5.6</td>
<td>5.1±5.9*</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. The test pulse was always preceded by prepulse at −110 mV for 2 seconds to eliminate the fast recovery of hyperpolarization before test pulse. Values represent the percentage of sodium current recorded after unblock train in the presence of flecainide (6 μM) for six preparations.

*Significance of difference between −100 mV and each of all potentials with p<0.05 by analysis of variance.
Voltage-dependent recovery mainly due to activation unblock. Since recovery from block reflects closed-state unblocking and activation unblocking,\(^{19}\) we attempted to define the separate mechanisms for fast and slow components of recovery. If the initial fast component is caused by closed-state unblocking, then this recovery process must continue when clamping to a depolarized potential, as long as the latter potential does not inactivate drug-free channels. On the other hand, if voltage dependence of recovery from block results from development of availability for activation unblock during the test pulse, then the conditioning depolarization before activation could diminish this availability (for a detailed description see Snyders and Hondeghem\(^{19}\)).

These two possibilities were tested in the experiment shown in Figure 6. After induction of block in the presence of 6 \(\mu\)M flecainide, the preparation was hyperpolarized for 2 seconds, and after a variable conditioning time at \(-100\) mV, a test pulse was applied. The potential of \(-100\) mV was selected because in this preparation it was near the top of the availability curve \((h)\) of the drug-free channels and at the bottom of the availability curve \((h')\) for activation unblocking. As the conditioning time at \(-100\) mV increased, the difference of recovery between \(-100\) mV and the hyperpolarized potentials \((-140\) and \(-160\) mV) declined until \(I_{Na}\) returned to the same level observed at \(-100\) mV. Thus, in about 200 msec, \(I_{Na}\) appeared as if there had never been a hyperpolarization. In three preparations, the apparent unblock by a 2-second hyperpolarization to \(-180\) mV completely reversed on clamping to \(-100\) mV \((\tau=98\pm12\) msec) Similar observations were also obtained in two other experiments using a 10-second hyperpolarization to \(-180\) mV.

**Time and voltage dependence of availability for activation unblock.** To determine the time and voltage dependence of inactivation over a wide range of potentials, the pulse protocol shown in Figure 7 (top) was used. Briefly, a fast pulse train was applied to elicit maximal block and was followed by a 2-second hyperpolarization to \(-200\) mV to maximize removal of inactivation. Test pulses were applied after a

---

**FIGURE 4.** Graphs showing contribution of use-dependent unblock to recovery from block. Use-dependent unblock of maximum upstroke velocity \((V_{max}:\text{left panel})\) and sodium current \((I_{Na}:\text{right panel})\) with 5 Hz train at different holding potentials \((-160, -140, \text{and} -120\) mV) was shown compared with the standard single-test pulse recovery protocol. \(\tau_{-100}\) is the time constant at \(-100\) mV. In both experiments, a 5 Hz train of pulses was applied instead of a single test pulse. All test pulses were plotted in one figure resulting in conclusive comparison of a single pulse (filled symbols) with multiple pulses (open symbols). Application of train relieved the block faster than a single pulse, even when associated with long recovery (15 seconds).

**TABLE 3. Voltage-Dependence of Percent Unblock per Activation**

<table>
<thead>
<tr>
<th>Potential (mV)</th>
<th>(V_{max})</th>
<th>(I_{Na})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-120)</td>
<td>46.0±4.6*(^{(4)})</td>
<td>10.8±2.3(^{(7)})</td>
</tr>
<tr>
<td>(-100)</td>
<td>25.7±5.3(^{(6)})</td>
<td>9.2±2.9(^{(7)})</td>
</tr>
<tr>
<td>(-90)</td>
<td>16.3±4.3 (^{(4)})</td>
<td>4.6±2.1(^{(7)})</td>
</tr>
<tr>
<td>(-85)</td>
<td>19.7±8.1(^{(6)})</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. Percent unblock per pulse was calculated as the index of contribution of a single pulse in recovery process (see text). Values represent the percentage of maximum upstroke velocity \((V_{max})\) or sodium current \((I_{Na})\) recorded after unblock train in the presence of flecainide. Numbers between parentheses indicate number of experiments. Analysis of variance was used for critical difference among multiple means.

*Significance of difference between \(-120\) mV and each of all potentials with \(p<0.01\).

†Significance of difference between \(-100\) mV and \(-90\) mV with \(p<0.05\).

‡Significance of difference between \(-120\) mV and each of other potentials with \(p<0.01\).
variable conditioning time at several potentials. The $I_{Na}$s during the test pulses were then normalized to the $I_{Na}$ observed immediately after the 2-second hyperpolarization. Under control conditions, $I_{Na}$ did not decline until the membrane potential was made more positive than $-100$ mV (Figure 7, bottom left). As the conditioning potential was made more positive, development of inactivation became more marked, and it developed increasingly faster. In the presence of flecainide (6 μM), availability for unblocking started to decline at potentials more positive than $-120$ mV, and this decline also became more marked and occurred more quickly with increasing depolarization. Control and drug data were fitted with single exponentials, and the time constants obtained from 12 experiments are summarized in Figure 8. The circles were obtained from reduction in availability (similar to the experiment shown in Figure 7); the squares were derived from development of availability (similar to Figure 3, bottom). The time constants exhibit the classic biphasic dependence on membrane potential both in control (Figure 8, top) and following block by flecainide (Figure 8, bottom). However, in the presence of flecainide the relation is shifted to more negative potentials and has a broader appearance.

To test whether the voltage dependence of unblocking is also primarily caused by activation unblock at 37°C, we did similar experiments using $V_{max}$. In three experiments using a 3-second hyperpolarization to $-110$ mV, on returning to $-70$ mV (near the top of the availability curve at this temper-

**Table 4. Effects of 3 μM Flecainide on h- and h'-Curves**

<table>
<thead>
<tr>
<th></th>
<th>$h$-Curve</th>
<th>$h'$-Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Flecainide</td>
</tr>
<tr>
<td>$V_{max}$ (37°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope factor (mV)</td>
<td>4.6±1.3 (7)</td>
<td>4.9±0.8 (8)</td>
</tr>
<tr>
<td>$E_{1/2}$ (mV)</td>
<td>$-56.5±5.7$</td>
<td>$-60.7±2.9$</td>
</tr>
<tr>
<td>$I_{Na}$ (17°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope factor (mV)</td>
<td>5.2±0.4 (4)</td>
<td>5.9±0.5 (7)</td>
</tr>
<tr>
<td>$E_{1/2}$ (mV)</td>
<td>$-81.5±7.9$</td>
<td>$-84.6±5.0$</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. $h$-Curve, voltage-dependent availability of normal sodium channel; $h'$-curve, voltage-dependent availability for activation unblocking of drug-associated sodium channel; $E_{1/2}$, membrane potential for half maximum value of $h$- or $h'$-curve. Slope factor and $E_{1/2}$ are calculated by using a least-square error fit to the Boltzmann equation. Numbers in parentheses indicate number of experiments. Standard $h$-curve was recorded before and 10 minutes after drug application, and $h'$-curve was then recorded in the presence of flecainide.

*Significance of difference by analysis of variance with $p<0.001$ among standard $h$-curves and $h'$-curve.
tions.

interpulse
shorter

Instead, block developed
appeared
in
(Figures
1

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Vol
66,
No
3,
March
1990

unblock similarly dis-
appeared in less than 200 msec.

Discussion

The present results show that in the presence of
flecainide, there was little tonic block of \( V_{\text{max}} \) or \( I_{\text{Na}} \). Instead, block developed in a use-dependent fashion (Figures 1 and 2) and was more pronounced at a shorter interpulse interval and at less negative potentials but was similar for long and short depolarizations. In addition, during prolonged depolarizations at 37°C there was a slow component of block develop-

opment, which was not observed at 17°C. This slow component appears to have kinetics and voltage-
derpendent features reminiscent of slow inactivation,\(^{32}\) which was not characterized in the present study.

Recovery from block was strongly voltage depen-
dent; that is, the extent of \( V_{\text{max}} \) recovery at more negative potentials exceeded that at more positive potentials (Figure 3). However, except during the initial phase, the overall rate of recovery exhibited little dependence on voltage (Table 2). In addition, unblocking was greatly enhanced by pulses from

Figure 6. Graph showing loss of availability for unblocking. The open symbols represent standard recovery at -100 (open circles), -140 (open triangle), and -160 mV (open square) in the presence of 6 \( \mu \)M flecainide (see left inset). The filled symbols represent data obtained following the pulse pattern shown in the right inset. After a 2-second hyperpolarization (-160 and -140 mV), the membrane potential was clamped back to -100 mV for various times, and a constant test pulse was used to monitor the decline of availability of the sodium current (\( I_{\text{Na}} \)). On clamping to -100 mV after a 2-second pulse to -140 or -160 mV, \( I_{\text{Na}} \) did not continue to recover, instead it declined as a function of time until it reached the level obtained at -100 mV.

Figure 7. Graphs showing time and voltage dependence of development of inactivation in drug-free (left panel) or blocked (right panel) channels. The pulse protocol is shown at the top. To obtain virtually complete block of the channels during the fast pulse train, 6 \( \mu \)M flecainide was used. Maximum availability of unblocking was then induced by a 2-second pulse to -200 mV. The decline of this avail-
ability was then induced by clamping to various potentials for various times and followed by a fixed test pulse. All values are normalized to maximum availability and plotted in a semilogarithmic scale. In the presence of drug, voltage dependence of inactivation appeared shifted toward more negative potentials. \( I_{\text{Na}} \), sodium current.
more negative potentials: use-dependent unblock (Figure 4). Kinetics and amplitude of use-dependent unblocking were strongly dependent on holding potential, interpulse interval, and temperature. Such voltage- and time-dependent effects often result when the affinity of the drug for the sodium channel receptor is modulated by the state of the channel.12

State-Dependent Affinity of Flecainide for Sodium Channels

Low affinity for rested channels. As with most antiarrhythmic agents,12 flecainide has a low affinity for the rested state of the sodium channel. Indeed, there was only a small reduction of \( V_{\text{max}} \) and no detectable reduction of \( I_{\text{Na}} \) when holding at negative potentials where the rested state is prevalent. The small amount of tonic block (5.8±4.9%) observed at 37°C when holding at −90 mV may partly result from block of slow inactivated channels or activation block that developed during the upstroke of the test pulse but before reaching \( V_{\text{max}} \). In any event, if flecainide does block rested channels, it must do so to a very small extent at concentrations below 3 μM.

High affinity for activated and low affinity for inactivated channels. Long and short depolarizations both induce the activated state of the sodium channel, followed by a long or short period in the inactivated state. For drugs that block inactivated sodium channels,33 block during long depolarizations exceeds that for short depolarizations. Since flecainide blocked sodium channels to the same extent during long and short depolarizations, use-dependent block by flecainide must not be associated with block of inactivated channels. Therefore, our results strongly suggest that, except for very long depolarizations, most of the use-dependent block by flecainide is associated with activation.

Mechanisms of Unblocking

Recovery of \( V_{\text{max}} \) and \( I_{\text{Na}} \) consisted of a strongly voltage-dependent early component, which was followed by a slower phase that appeared less voltage dependent.

Slow voltage-independent component of unblock is \( RD \Rightarrow R \) and \( ID \Rightarrow I \). At 37°C the slow recovery phase had a time constant of about 10 seconds, which is similar to that observed in multicellular preparations at a physiological temperature.2,3 This recovery was very slow at 17°C (we did not fully characterize this slow phase at 17°C as it required minutes for completion). For both \( V_{\text{max}} \) and \( I_{\text{Na}} \) this slow component appeared to be virtually independent of voltage. No major change in rate of recovery was observed between −100 and −75 mV for \( V_{\text{max}} \) (Figure 3, top panel) nor between −200 and −120 mV for \( I_{\text{Na}} \) (Figure 3, bottom panel). Since, over this potential range, drug-associated channels move between RD and ID states (Figures 5 and 6), it follows that for flecainide RD⇒R and ID⇒I cannot have very different kinetics and that the rate constants controlling this process cannot be very voltage dependent. Actually, when doing recovery experiments where the contributions of activation unblock are minimized by a depolarizing prepulse (such that RD⇒ID but not R⇒I occurs), the slow recovery from block became independent of voltage (Table 2).

Fast voltage-dependent component of unblock is \( AD \Rightarrow A \). The early phase of recovery was markedly voltage dependent. As a result, use-dependent unblocking from block by flecainide was enhanced by hyperpolarization (Figures 2, 4, and 5) until a plateau was reached near −110 mV (\( V_{\text{max}} \)) or −160 mV (\( I_{\text{Na}} \)). This is qualitatively similar to previous observations for O-demethyl encainide (\( V_{\text{max}} \), \( I_{\text{Na}} \)) and quinidine (\( I_{\text{Na}} \)): the voltage dependence of availability for activation unblocking (\( h' \)-curve) was shifted by −16 mV at 37°C and by −34 mV at 17°C compared with availability of drug-free sodium channels (\( h \)-curve), and its slope was reduced 2.0-fold at 37°C and 2.3-fold at 17°C (Table 4).

Hyperpolarization promotes the transition from the inactivated drug-associated state to the rested drug-associated state (RD⇌ID).10,11,16,17,19 During the subsequent activation, activation block (A⇒AD) and unblock (AD⇒A) occur14–19:

\[
\frac{dA}{dt} = -k_A \cdot [D] \cdot A + I_A \cdot AD
\]

where \( D \) is the drug-associated state. At steady state (\( dA/dt = 0 \)):

\[
I_A/(k_A \cdot [D]) = A/AD
\]

![Graph showing voltage dependence of the time constant (τ) for development of inactivation (squares) and recovery from inactivation (circles). The top panel was obtained from seven cells under control conditions; the bottom panel was from seven cells in the presence of 6 μM flecainide. The curves are obtained from procedures obtained in experimental protocols similar to those described in Figure 3, bottom, whereas the squares are obtained from experimental procedures similar to those described in Figure 7. The curves represent a spline fit through the mean of the data points.](image-url)
Thus, when the occupation of the R pool is relatively large so that on activation the A pool exceeds the steady-state equilibrium described by Equation 2, activation block occurs; when the RD pool is relatively large so that on activation the AD pool exceeds the equilibrium, activation unblock occurs (see Figure 2). When virtually all channels are blocked (RD, AD, and ID), the \( I_{Na} \) that results on activation (RD\( \rightarrow \)AD\( \rightarrow \)A) becomes an indicator of the RD occupancy just before activation (first pulse in Figure 5). Based on this interpretation, it is clear that fleca-

inide-blocked channels become available (ID\( \Rightarrow \)RD) over a potential range of \(-100 \text{ mV}\) to \(-160 \text{ mV}\) at \(17^\circ C\) and over the \(-65\) to \(-100 \text{ mV}\) range at \(37^\circ C\). It is important to note that over this potential range the standard availability curve of drug-free channels (open circles in Figure 5) even in the presence of the drug (filled circles) was already maximal. In addition to this negative shift, the slope of the availability curve (\( h' \)-curve) is also much shallower than in control (Table 4).

To guard or to modulate. The reduced availability and hyperpolarized shift by flecaainide could result

![Graph showing mean voltage and time dependence of closed-state transitions in drug-free and drug-associated channels.](image)

**Figure 9.** Graphs showing mean voltage and time dependence of closed-state transitions in drug-free and drug-associated channels. Top panel: Mean availability of sodium channel \( (I_{Na}) \) in the absence of drug (dashed line) and relative availability for unblocking of blocked channels (solid line) using values of Table 4. Middle panel: Mean for development of inactivation and recovery from inactivation (1/\( \tau \) from Figure 8, where \( \tau \) is the time constant). Bottom panel: The voltage dependence of the rate constant for development of inactivation (\( \beta \)) and recovery from inactivation (\( \alpha \)) were computed using the steady-state availability and its mean rate constant. The open circles represent the mean of all control values; the solid circles represent the mean in the presence of 6 \( \mu M \) flecaainide. Curves represent single-exponential fits to the data. These curves are defined by the functions as follows: \( \alpha = 0.60 \ e^{-V_{m}0.36} \), \( \beta = 9500 \ e^{5V_{m}0.9} \), \( \alpha' = 0.31 \ e^{-V_{m}0.36} \), and \( \beta' = 771 \ e^{5V_{m}10} \), where \( V_{m} \) is the membrane potential.
from altered voltage dependence of channel gating (the modulated-receptor theory) or from a voltage-dependent rate of interactions without alteration of gating (the guarded-receptor hypothesis). From the voltage dependence of the average availability of sodium channels (Table 4 and Figure 9, top panel) and the mean time constant (Figure 8 and Figure 9, middle panel) one can compute the voltage dependence of the rate constants for development of inactivation (β) and recovery from inactivation (α) by the procedure described by Hodgkin and Huxley. This analysis (Figure 9, bottom panel) clearly shows that for drug-associated channels transitions (α' and β') between the rested and inactivated states (RD⇌ID) occur at more negative potentials than those (α and β) for drug-free channels (R⇌I).

It could be argued that such alteration results from isochronal, but nonsteady state, analysis. We do not believe so, because the closed state unblocking for flecainide is so slow, compared with the gating kinetics, that the isochronal analysis must provide a good approximation of the true voltage dependence of inactivation gating. More important is the experiment shown in Figure 6. If voltage dependence of drug unblocking was not the result of altered gating of blocked channels, then on clamping to −100 mV, recovery should monotonically continue, albeit at the slow rate characteristic of −100 mV. Instead, after the apparent unblock during hyperpolarization, block appeared to redevelop while holding at −100 mV. The latter is not the result of a high affinity for the drug at this potential, because maintained holding potential at −100 mV leads to full unblocking with a very slow time constant. Indeed, the effect of the hyperpolarization dissipated quickly (τ=98±12 msec), after which the slow recovery process typical for −100 mV became visible again. This biphasic behavior cannot be accounted for by a set of voltage-dependent rate constants for unblocking as used by the guarded receptor.

This result is easily accounted for in terms of the modulated receptor theory. After induction of extensive block, a strong hyperpolarization quickly eliminates inactivation (ID⇌RD). If the test pulse is applied from hyperpolarized potentials, then the blocked channels activate (RD⇌AD). Since under this situation AD is occupied beyond the equilibrium of Equation 2, activation unblock far exceeds activation block (few channels in A) resulting in net activation unblock and a sodium current: AD⇌A. If, however, the test pulse is preceded by a pulse to −100 mV, then, because of the altered voltage dependence of gating (Figure 9), the drug-associated channels inactivate (RD⇌ID) with a time constant of 98±12 msec and availability for unblocking declines. Since the loss of availability for unblocking (RD⇌ID) exceeds closed-state unblocking (RD⇌R and ID⇌I), recovery of flecainide appears to decline. If the hyperpolarization-induced availability for unblocking is fully dissipated, then the slower closed-state unblocking becomes visible again (see slow increase of INa during recovery time between 2.2 and 2.4 seconds in Figure 6). Gintant and Hoffman have also shown that for quaternary lidocaine analogues (QX-314 and QX-222), prepulse hyperpolarization could induce more recovery from block if the test pulse followed shortly after the hyperpolarization. The above results support the modulated-receptor proposal that activation unblocking is voltage and time dependent primarily because drug-associated channels (like drug-free channels) exhibit inactivation and recovery from inactivation. However, the transitions between rested and inactivated states occur at more negative potentials for drug-associated channels than for drug-free channels. Voltage dependence of the A⇌AD transitions has been also proposed but was not studied in the present experiments.

It could be argued that strong hyperpolarization may alter the protonation of the drug and consequently speed up recovery from block. This argument would not apply for the quaternary compounds (QX-314 and QX-222) used by Gintant et al. Moreover, since the drug on the receptor appears to be more under the influence of external pH than internal pH, hyperpolarization would attract H+ and, thus, lower the pH at the channel receptor. This would promote the charged form of the drug and should slow recovery from block by trapping. Thus, since drug occupancy modifies channel gating and since recovery from block is not necessarily monotonic, our results are incompatible with the guarded-receptor hypothesis but conform to modulated-receptor principles.

In summary, recovery from flecainide block is strongly dependent on holding potential mainly because availability for fast unblock during activation (RD⇌AD⇌A) is controlled by membrane potential. In contrast, the slow unblocking from the closed states (RD⇌R and ID⇌I) appears to have little voltage dependence.

Temperature

Cooling dramatically slows the closed-state interactions of antiarrhythmic drugs. In a recent study, Johns et al. showed that, on cooling below 20°C, closed-state unblock (RD⇌R and ID⇌I) from use-dependent block by O-demethyl encainide was completely abolished: even when waiting for 1 hour, there was no measurable recovery from block. Although not as extreme, similar slowing of recovery from block has been observed by numerous investigators. The present results provide another example of alteration of drug action by cooling. Therefore, studies to elucidate mechanism of antiarrhythmic drug action must include experiments at 37°C. Indeed, cooling may alter partitioning of tertiary amines in the lipophilic phase of the membrane. As a result, the alteration of drug action by cooling need not be only a quantitative one.

Perhaps the most dramatic effect of reduced temperature is the enhancement of the negative voltage shift of the availability for unblocking asso-
ciated with activation. Consequently Johns et al.\(^8\) interpreted this to indicate that the drug and cooling increase the free-energy difference and the barrier between the RD and ID states, so that at certain potentials recovery from inactivation is less likely and occurs more slowly. Our results are compatible with this interpretation.

Clinical Implications

Our results clearly demonstrate that unblocking from closed states is a relatively minor route for unblocking of flecainide. Indeed, the slow recovery processes\(^2,3\) \((\tau \approx 10–20\) seconds\) yields too little unblocking during one diastolic interval. For example, for a normal diastolic interval of about 500 msec, less than 3% of blocked channels can unblock. Instead, fast use-dependent unblocking appears much more important. Indeed, at \(-90\) mV approximately 16% of blocked channels unblock per activation (Table 3), and thus, at \(-90\) mV activation unblock is five times more important than the slow diastolic recovery process. For shorter cycle lengths the relative importance of activation unblock would become even more significant.

On depolarization, the importance of activation unblock progressively declines. As a result, for pulses of fixed duration and to fixed potentials, as the resting potential becomes less negative, the level of block increases (e.g., Figure 1). Since the slow unblocking process is too slow and has a voltage dependence too shallow to account for these differences, by elimination they must result from the steep voltage dependence of activation unblock. Although there must be more activation block at \(-90\) than at \(-65\) mV (block is proportional to channels available for block; see Equation 1), the fact that \(V_{\text{max}}\) at \(-90\) mV is nevertheless larger than at \(-65\) mV mandates that activation unblocking at \(-90\) mV must be much larger than at \(-65\) mV. The latter conclusion is clearly supported by Figure 5.

Use-dependent unblocking as observed here endows flecainide with at least two potentially useful features. First, the drug will have more effect against tachycardias than normal heart rates. Indeed, at fast heart rates less time for development of availability for unblocking is present during diastole (ID\(\rightarrow\)RD). Second, the drug will also have more effect in depolarized than in normal tissue because availability for unblocking develops less in depolarized tissue (see Figures 5–7). Tissues at resting potential near \(-90\) mV will clearly exhibit much more activation associated unblocking than depolarized tissues. Since arrhythmias are frequently associated with depolarization, flecainide can by this means markedly depress conduction in the depolarized tissue responsible for arrhythmias, while interfering less with the conduction in normal tissue.

However, it should be noted that, in the absence of significant slow diastolic recovery, small changes in membrane potential will markedly alter the activation unblock. As a result, a therapeutic concentration at a given heart rate and membrane potential can easily become a toxic one at increased heart rates or serum potassium.\(^1\) This danger of agents that have slow time constants for diastolic unblock was previously discussed in detail\(^8\) and may be, in part, responsible for the increased sudden death by flecainide in a recent clinical trial.\(^9\)

Conclusion

Our results show that flecainide binds primarily to the activated state of cardiac sodium channels. Recovery from block by flecainide consists of a fast and a slow process. The slow component is due to unblocking during closed states and is not very voltage dependent. The fast component is strongly voltage dependent and results from unblocking associated with activation. Availability for activation unblocking increases with hyperpolarization and decreases with depolarization, and its voltage dependence is shifted to more negative potentials and has a shallower slope than availability for activation in drug-free channels. These results are compatible with the modulated-receptor theory but not with the guarded-receptor hypothesis.

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KEY WORDS • flecainide • antiarrhythmic agents • cardiac sodium channels • use-dependent block and unblock
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