Effects of Quinidine on the Sodium Current of Guinea Pig Ventricular Myocytes
Evidence for a Drug-Associated Rested State With Altered Kinetics

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In guinea pig cardiac myocytes quinidine (20 μM) caused <10% tonic block reduction of the sodium current at −120 mV, but a fast pulse train reduced it more than 90%. Recovery from use-dependent block was time and voltage dependent, and was always slow (τ=34±10 seconds at −160 mV; τ=90±35 seconds at −120 mV; n=15, mean±SD, p<0.001, paired t test). However, in association with repeated activation a fast component of recovery from block was observed: use-dependent unblocking. Availability of sodium channels for use-dependent unblocking was enhanced by hyperpolarization until a plateau was reached near −160 mV. Compared with the availability of drug-free sodium channels (h-curve), the voltage dependence of availability for use-dependent unblocking (h'-curve) was shifted by about 30 mV to more negative potentials, and its slope was reduced 2.5-fold. At −160 mV, the kinetics of development of availability of sodium channels for use-dependent unblocking were rapid (τ<10 msec). Depolarization to −120 mV reduced the availability of sodium channels for fast unblocking with a time constant of 191±46 msec (n=14). Finally, block established by frequent brief depolarizations (activations) declined during prolonged inactivation. From these results we concluded that the time and voltage dependence of the availability of sodium channels for unblocking are considerably different from the availability for activation of drug-free channels, that rested drug-associated channels do exist, and that drug-associated channels do not conduct (or at least have a greatly reduced conductance) upon activation unless they first unblock. Furthermore, activated and inactivated channels have a different affinity for quinidine, and since quinidine can occupy the channel receptor even when "guarded," our results are incompatible with the guarded receptor hypothesis but can be explained within the framework of the modulated receptor hypothesis. (Circulation Research 1990;66:565–579)

Quinidine reduces the sodium conductance of cardiac cells in a voltage-1 and time-dependent2,3 fashion. These actions of quinidine on the cardiac sodium conductance were demonstrated by measurement of the maximum upstroke velocity of the cardiac action potential and have been interpreted in terms of the modulated receptor hypothesis.4 Briefly, sodium channels can exist in three primary states (see Figure 1): 1) The rested (R) state is a closed state from which channels can be opened by depolarization and is most prevalent at negative membrane potentials; 2) the inactivated (I) state is a closed state from which channels cannot be opened by depolarization and is most prevalent at depolarized membrane potentials; and 3) the activated (A) state occurs transiently upon depolarization and is closely associated in time with channel opening.6 In each of these states the channels can bind drug to form the corresponding RD, AD, and ID states, interactions that are governed by state-characteristic association (k) and dissociation (l) rate constants. Drug-associated channels are assumed not to conduct sodium and to have their voltage dependence of inactivation shifted to more negative potentials.4,7,8 In the present study, we wanted to test these interpretations by use of a more direct measurement of the sodium conductance: determination of the sodium current under voltage-clamp conditions.

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A set of modulated receptor rate constants for quinidine can satisfactorily predict the effects of therapeutic concentrations of quinidine over a wide range of driving rates, for a broad range of extrasynto-

toles, and over an extensive range of holding potentials. In the latter study, the authors found no use-dependent blocking in the presence of large doses of quinidine (16 μg/ml) at -120 mV even at fast heart rates (3.3 Hz). Therefore, they concluded that recovery from block must proceed very quickly at potentials more negative than -108 mV and interpreted their results as indicative of fast transition from RD→R (Figure 1). Limitations of the sucrose gap technique precluded reliable measurements of the time constant for recovery from block at these hyperpolarized potentials, mainly because of the large and damaging currents required. More recently, voltage-clamp experiments in ovine Purkinje fibers indicated that the time constant of recovery from block by quinidine becomes only slightly shorter with hyperpolarization over the range of -55 to -100 mV. Therefore, either the time constant of diastolic recovery from block has to shorten considerably at potentials negative to -100 mV, or the lack of use-dependent blocking at -120 mV is an artifact, possibly due to shortcomings of the experimental technique (V_{max} in sucrose gap), or sodium channels exhibit an additional mechanism for recovery from block not revealed in experimental protocols that assess diastolic recovery.

To differentiate among these possibilities, we determined the time constants for recovery from use-dependent blocking by quinidine over a wide range of membrane potentials and found that even upon hyperpolarization to -160 mV, the time constants for recovery from block remained rather large (τ>30 seconds). Moreover, little use-dependent blocking was observed at potentials more negative than -160 mV. Actually, under appropriate conditions we observed the opposite phenomenon: use-dependent unblocking. This suggested a mechanism for fast recovery from block associated with channel activation (AD→A), in addition to the slow diastolic recovery processes (ID→I and RD→R). The primary goal of the present study was characterization of the time and voltage dependence of this fast recovery process. Preliminary results from this study have been published previously.

**Materials and Methods**

**Cell Preparation**

Experiments were performed on single ventricular myocytes obtained from adult guinea pig hearts by use of an enzymatic dispersion technique. The solution used for the isolation was a Joklik-modified minimum essential medium (Gibco, Grand Island, New York) buffered to pH 7.35 with 25 mM NaHCO₃ and 5% CO₂. The hearts were mounted on a Langendorff perfusion apparatus and rinsed for 1 minute with this nominally calcium-free solution, whereupon they were exposed for 15 minutes to the same solution supplemented with collagenase 200 units/ml (Worthington type II, Cooper Biomedical, Malvern, Pennsylvania) and 50 μM calcium. The ventricles were cut down, minced, and incubated for another 10 minutes in the enzyme-containing solution. After filtration through a 250-μm nylon mesh, the dispersed cells were washed three times by centrifugation and resuspension in enzyme-free solution containing 0.1% bovine serum albumin (Sigma Chemical, St. Louis, Missouri) and 1 mM calcium. The cells were then suspended in Medium 199 (Sigma Chemical) containing 1 mM calcium and stored in an incubator at 37° C until used (within 12 hours).

**Solutions and Drugs**

The extracellular solution used in the experiments contained (mmol/l) NaCl 20, CsCl 110, CaCl₂ 1, MgCl₂ 2, CoCl₂ 3, glucose 10, and HEPES 10, adjusted to pH 7.35 (22° C) with approximately 2 mM
CsOH. The intracellular solution contained (mmol/l) NaF 10, CsF 110, CsCl 20, MgCl2 2, EGTA 2, and HEPES 10, adjusted to pH 7.2 (22°C) with approximately 4 mM CsOH. Addition of 3 mM Co reduced the sodium current by 20–30%, but this reduction did not exhibit use dependence. Similar observations have been made for a number of divalent cations.15 In all experiments, unless explicitly stated otherwise, the concentration of quinidine (quinidine sulfate, Aldrich, Milwaukee, Wisconsin) was 20 μM. The quinidine was added to the extracellular solution from an 8-mmol/l stock solution.

Electrophysiological Techniques

Experiments were performed in a small (0.5-ml) tissue bath mounted on the stage of an inverted microscope (model TMS, Nikon, Garden City, New York). An aliquot of cell suspension was released into the bath. The cells were allowed to settle and adhere to the bottom for 10 minutes, whereupon the chamber was perfused continuously (flow rate 0.5–1.0 ml/min). The bath was cooled to 17±1°C by a Peltier device (Midland Ross, Cambridge, Massachusetts). The temperature drift was less than 0.5°C for all experiments.

Microelectrodes were pulled from coagulation capillary tubes (Fisher Scientific, Pittsburgh, Pennsylvania) on a horizontal puller (Sutter Instrument, San Rafael, California) and heat polished with a microforge (Narashige, Tokyo, Japan).

The results presented were obtained from 44 successful experiments in which the whole-cell voltage-clamp configuration of the patch-clamp technique16 was used. Small cells were selected (87±15×19±3 μm; n=44), and in the last 17 experiments where cell capacitance (integration of uncompensated capacitive transients) was measured, it averaged 71±14 pF. Only cells that were rod shaped, had clear cross-striations, and remained quiescent were used for study. Additionally, in a separate study, cells prepared by use of the same method were shown to have a resting potential of −84.5±3 mV (n=14) when superfused with standard Tyrode’s solution (Kc=4 mM). Only microelectrodes with a resistance less than 1 MΩ (0.65±0.16 MΩ) were used. All measurements were done with the Axopatch-1A patch-clamp amplifier (Axon Instruments, Burlingame, California). After formation of a seal between the electrode and the cell (10±4 GΩ), the electrode capacitance was compensated by an analog circuit and the patch was disrupted with slight additional suction. Cell capacitance and series resistance were compensated by analog circuits. Leak subtraction was mainly done by use of an analog circuit during the experiment; some additional digital correction was performed during analysis by subtraction of scaled averaged tracings from small depolarizations that resulted in no activation of the sodium current. Voltage steps were provided by a 12-bit digital-to-analog converter (LabMaster, Scientific Solutions, Solon, Ohio), controlled by the pClamp software package (Axon Instruments). To provide more sophisticated pulse patterns and data acquisition modes, we built a stimulator based on two programmable arbitrary waveform generators (Wavetek, San Diego, California), controlled by custom software developed in ASYST (MacMillan Software, New York). The different voltage-clamp protocols are illustrated in the insets of Figures 2 through 6. Currents were filtered at 5 kHz (−3 dB; four-pole Bessel filter), sampled at 20 kHz by use of a 12-bit analog-to-digital converter, and saved on disk or digital tape for subsequent analysis.

Limitations of Methods

We took great care to select small cells, to use low-resistance electrodes, to establish good seals, and to reject cells that had maximum sodium currents in excess of 10 nA. In addition, the capacitance was subtracted as accurately as possible with a three-time-constant adjustment. Series resistance was carefully compensated until the voltage clamp showed signs of instability. At that time the series resistance compensation was slightly reduced, and the final compensation was usually about 70%.

After compensation, the effective series resistance was close to 0.4 MΩ, and since the peak sodium current was less than 10 nA, the maximum voltage error across the electrode tip should have been less than 4 mV except for brief transients while the amplifier was charging the cell capacitance (see below). In the presence of drug the sodium currents were frequently much smaller, and the error across the access resistance was accordingly smaller. The mean cell capacitance of 71 pF, combined with a maximum access resistance of 0.4 MΩ, yields a maximum time constant of 28 μsec for charging the cell membrane. The observed time constants confirm the above calculations, as the capacitance transient was usually complete in less than 100 μsec. Only for very large depolarizations, which generate large capacity transients combined with fast kinetics of the sodium current, did the capacity transient and sodium current overlap. Although we did use such pulses for conditioning, for the test pulses we always clamped to −20 mV (near the peak of the current-voltage relation), where the capacity transient and the sodium current were clearly separated. In all cells, the current-voltage relation had a smooth descending limb that spanned at least 30 mV from threshold potential to the maximum inward current. Furthermore, in the absence of drug, the currents obtained for h-curves (availability curves for sodium channels) could be scaled for superimposition. Since large sodium currents would be expected to be less adequately controlled than small ones, such superposition would not occur in the absence of adequate control.

Data Analysis

Analysis of the experimental records was performed off-line on an IBM AT computer using custom software, written in FORTRAN and ASYST. All curve fitting was done with a nonlinear least squares
error search algorithm (modified Gauss-Newton) to the indicated functions.

Exponentials were of the form

\[ y = \sum_{i=1}^{n} A_i e^{B_i x} + C \quad (n=1, 2, \text{or} 3) \]

where A represents amplitude, B represents rate constant, and C represents baseline. Double exponentials were accepted as the fit of choice (over monoexponential ones) if the residual variance was significantly reduced (F test).

Boltzmann equations were of the form

\[ y = \frac{1}{1 + e^{(E-E_{1/2})/k}} \]

where E is the membrane potential, \( E_{1/2} \) is the membrane potential of midpoint of curve, and k is the slope factor.

All data are expressed as mean±1 SD. Comparisons between two means were done by use of Student's t test. Comparisons among multiple means were done by analysis of variance (ANOVA) and Scheffe's test for critical difference. Statistical significance was set at \( p<0.05 \).

Results

Tonic and Use-Dependent Block by Quinidine

The primary aim of the present study was characterization of the time and voltage dependence of drug-associated channels and comparison of these values with those of drug-free channels. Thus, it was necessary to achieve both maximal and minimal block. Tonic block is the drug-induced reduction of the sodium current that persists after a long rest period and is usually the minimum level of block that can be achieved in the presence of drug.\(^{17}\) Upon application of depolarizations the level of block usually increases (use-dependent block).\(^{17}\) Hence, the optimal drug concentration for the present experimental purpose should yield minimal tonic block and maximal use-dependent block.

At a holding potential of \(-120\) mV, in 5 \( \mu M \) quinidine the tonic block was so small that it commonly could not be detected; in 20 \( \mu M \) quinidine the tonic block was noticeable but small (<10%), while in two experiments at 50 \( \mu M \) the tonic block exceeded 10% (11% and 20%).

With a twin-pulse technique, increases in the conditioning pulse duration increased the block very slowly or not at all.\(^{18}\) Actually, once block was induced, prolonged depolarizations reduced the level of block (see below). Thus, the twin-pulse technique was not a satisfactory way for induction of high levels of block under the present experimental conditions. In contrast, upon application of a train of pulses, block developed with each depolarization. The block developed more quickly and was more pronounced with shorter cycle lengths, stronger depolarization, higher drug concentration, and more positive holding potential, as long as this holding potential was negative enough (more negative than \(-100 \) to \(-120 \) mV) to make the drug-free channels available for activation (i.e., not inactivated). In 20 \( \mu M \) quinidine the sodium current could be reduced to 7±3% of control (\( n=17 \)) after two hundred 5-msec pulses from \(-120 \) to +30 mV at 10 Hz. In Figure 2, the sodium current after such a block-induction train in 20 \( \mu M \) quinidine is superimposed on a control sodium current. Both tracings were elicited by a depolarization to \(-20 \) mV. The control tracing shows maximum available sodium current (\( I_{Na} \)); the tracing in the presence of quinidine was obtained 100 msec after the last pulse of the conditioning train (i.e., the tracing matches the cycle length of the conditioning train but was obtained at \(-20 \) mV). In control conditions the similar tracings yielded more than 90% of the maximal current (see below). Thus, in the presence of 20 \( \mu M \) quinidine this 200-pulse-conditioning train reduced the peak sodium current to 4% of control. Although stronger depolarizations at faster rates might have produced the block more quickly or to a greater extent, production of block in excess of 90% in 20 seconds was considered adequate for the present study. Moreover, it was well tolerated by the preparations and reproducible throughout the experiment. In the remainder of this paper, this 200-pulse-conditioning train is referred to as the block-induction train. It was used whenever a substantial block by quinidine was desired.

Recovery From Block

After the block-induction train the sodium current eventually returned to its steady-state value. This recovery phenomenon resulted from three main processes: 1) In preparations where the block was incomplete (Figure 3), there was an early recovery process that had a time course very similar to the time course of
the repriming process in the absence of drug. Presumably this early phase represents removal of inactivation of drug-free sodium channels. 2) There was a slow phase of recovery in which rate was dependent upon the holding potential.\textsuperscript{19} At \(-160\) mV the slow phase of recovery from use-dependent blocking occurred faster (\(\tau = 34 \pm 10\) seconds) than at \(-120\) mV (\(\tau = 90 \pm 35\) seconds; \(p < 0.001\), paired \(t\) test, \(n = 15\)). Importantly, the slow phase of recovery from quinidine block, though significantly faster at more negative membrane potentials, remained slow: it required tens of seconds even at very negative potentials. 3) Much of the potential-dependent difference in amount of recovery from block developed early during the recovery interval. As a result the recovery process at \(-160\) mV (Figure 3) was shifted upward compared with that at \(-120\) mV. This fast unblocking phase was already present during the first few milliseconds of the recovery period and forms the main object of study of the present paper.

**Fast Recovery From Block**

In preparations in which the block-induction train achieved nearly complete block, the early recovery times represent mostly recovery by the fast unblocking process (no removal of inactivation of drug-free channels and recovery time too short for the slow process). In Figure 4 the block-induction train induced at least 97% block. Indeed, at \(-120\) mV drug-free channels exhibited reactivation that had a time constant in the range of 20–30 msec, so that reactivation was mostly complete in 100 msec (see open triangles connected by dotted lines in Figure 4). In the presence of quinidine, after the block-induction train at \(-120\) mV less than 3% of the sodium current reactivated in 100 msec (open triangles connected by solid lines). For increased resolution at short recovery intervals, the data are plotted on a logarithmic time scale. In this format, it becomes apparent that even for recovery times as short as 20 msec (leftmost open symbols in Figure 4), there was a voltage-dependent increment in the sodium current. For example, whereas there was virtually no sodium current 20 msec after the block-induction train at \(-120\) mV (<3% of control; open triangle), at \(-160\) mV (open circle) the current had an amplitude of 27% of control. Thus, fast unblocking of sodium channels resulted in a 24% increase of sodium current in 20 msec or less.

It is not clear whether this early recovery at \(-160\) mV developed early during the recovery interval, before activation, or whether the recovery was associated with activation itself. In the latter case, application of a train of brief pulses at various times after the block-induction train would induce recovery on a pulse-by-pulse basis. An appropriate pulse pattern for this test is shown in the inset of Figure 4. This complex figure contains three additional crucial features:

1) At early recovery times in the presence of quinidine, the increase in amplitude of the sodium current from the first pulse to the fifth pulse was large at \(-160\) mV (about 35%; difference between leftmost open and closed circles) and exceeded by far the minimal increase at \(-120\) mV (<3%; compare...
the leftmost open and closed triangles). The recovery induced by the five additional pulses cannot be accounted for by the extra time for recovery: in this 1-second period (five 5-Hz pulses) less recovery occurred (only 12%) in the absence of the short 5-Hz train. Also, to obtain the same amount of recovery in the absence of the pulses, one needed a 15-second recovery period at −160 mV. Thus, repeated activation of the channels resulted in relief of block (use-dependent unblocking).

2) At −120 mV the sodium current of the first pulse recovered to 36% in 60 seconds. Although in the experiment shown in Figure 4 the recovery period was not long enough to obtain full recovery from block, in four other experiments we observed that at −120 mV after a 2-minute recovery period the sodium current recovered in excess of 70%. With a time constant of 90 seconds, 5–10 minutes would be required to approach full recovery. In any event, the relative lack of recovery by the pulses at −120 mV cannot be ascribed to tonic block, since the block did dissipate, albeit slowly, at this potential.

3) As the five pulses were applied at later recovery times, the use-dependent unblocking declined until the time beyond which the test pulses induced block instead (use-dependent blocking). Thus, there existed one level of block at each potential (at 5 Hz) where development of and recovery from block balanced each other. This level of block is graphically represented as the intersection of the lines connecting the filled and open symbols for each holding potential. At −120 mV this level was at 95% reduction of the sodium current and was reached in about 300 msec of recovery after the block-induction train (levels indicated by arrows in Figure 4). At −160 mV this steady-state level was reached when the sodium current was reduced by only 14%, after a 50-second recovery interval. At −140 mV the steady-state level was reached when the sodium current was reduced by about 40% compared with control. If pulses were applied when the sodium current exceeded this steady-state level, block was induced, while below this steady-state level block was relieved. Similar results were observed in 14 experiments. During this 1-second train at 5 Hz virtually all blocking/unblocking was associated with activation, because there was too little time for the slow processes to contribute in an important way. Therefore, at this steady-state level of block, the amount of blocking and the amount of unblocking per activation must have approached equality.

Voltage Dependence of the Fast Recovery Process

Use-dependent unblocking was greater at −160 mV than at −140 or −120 mV (Figure 4). For more accurate determination of the voltage dependence of the availability of sodium channels for use-dependent unblocking, two pulse patterns were used. Availability curves obtained from sodium currents elicited after holding at a given potential for 5 seconds (Figure 5A, inset) will be referred to as h-curves. Determination of h-curves in the presence of quinidine was preceded by an unblocking train (eleven 5-msec pulses at 5 Hz from −160 to −20 mV) to minimize the fraction of blocked sodium channels.

In contrast to the above standard h-curves, which emphasize the behavior of drug-free channels, quantitation of the availability curves of drug-associated channels was also desired. For this purpose, block was maximized by a block-induction train (sodium...
current reduced to <10% in all 14 experiments shown in Figure 5A), and after a 1-second holding period at the desired potential (long enough for complete removal of inactivation but short enough for minimal unblocking by the slow process), a test pulse was applied (Figure 5A, inset). The resulting sodium currents will be referred to as h'-curves. In a few experiments shorter and longer holding periods were used, and these appeared to have little influence on the general shape of the h'-curves. In the 1-second holding period the slow recovery process contributed only 1–3% to the current (see "Discussion").

Both types of curves were fitted with Boltzmann equations, and their midpoints, slope factors, and mean differences are summarized in Table 1. Under control conditions, in all experiments the h- and h'-curves were virtually superimposed: their midpoints and slope factors differed by less than 1 mV. In Figure 5A the mean h-curve (top solid line) and the individual data points for all h'-curves (upper cloud of points) clearly show that h- and h'-curves closely resemble each other in control.

In the presence of quinidine (20 μM), the h- and h'-curves were very different: The h'-curve had a substantially more negative midpoint and a greatly reduced slope compared with the h-curve, and these differences were significant (p < 0.001; see Table 1). In Figure 5A, the h-curve (small dashed curve) and h'-curve (bottom solid line) are compared. The individual data for the h'-curves are represented by the bottom cloud of points. In four experiments it was possible to extend the h'-curves to -200 mV, and in each case the h'-curve achieved a plateau over the range of -160 to -200 mV. The h'-curve in the presence of quinidine was scaled to the amplitude of the control curves (large-dash curve). Obviously, even after normalization the h'-curve remained less steep and had its midpoint clearly shifted to more negative potentials, compared both with control and with the h-curve obtained in the presence of quinidine with the unblocking procedure (which yielded 85% unblock).

In a few experiments it was possible to wash out the quinidine, at least partially. In each case the slope returned toward control values, and the midpoints showed a similar trend. A complete reversal cannot usually be expected, partly because the quinidine may not completely wash out and partly because inactivation curves are known to drift to more negative potentials, especially during the first few minutes after breaking into the cell.20-22 It is important to notice that even in the presence of drift the midpoints of the h- and h'-curves after washout of the quinidine had midpoints that were substantially more positive than the midpoint for the h'-curve in the presence of quinidine (Table 1). Even in the presence of drift, the slope of the h-curves did not change significantly. Thus, the more negative midpoint and reduced slope of the h'-curves in the presence of quinidine cannot be explained by drift or possible deterioration of the preparation.

Furthermore, in some experiments this drift stabilized shortly after breaking into the cell, and further drift was negligible. Figure 5B depicts results in an experiment obtained in 50 μM quinidine and in which there was very little time-dependent drift of inactivation. The h-curves before, during, and after quinidine had slope factors of 5.6, 7.1, and 6.1 and midpoints of -98.2, -99.3, and -100.5 mV. In

### Table 1. Effects of Quinidine (20 μM) on h- and h'-Curves

<table>
<thead>
<tr>
<th></th>
<th>C-h</th>
<th>C-h'</th>
<th>Q-h</th>
<th>Q-h'</th>
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<td>35*</td>
<td>15†</td>
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<tr>
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<td>9</td>
<td>35*</td>
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<tr>
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<td>26*</td>
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<tr>
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<td>W-h'</td>
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Midpoints and slope factors are represented as mean±SD, expressed in millivolts. Differences between means and their statistical significance, as determined by analysis of variance, are represented immediately below in matrix format. C, control; Q, quinidine; W, washout of quinidine; E<sub>50</sub>, membrane potential of midpoint of curve; k, slope factor; h and h', sodium channel availability curves (see "Results"); n, number of experiments.

*<i>p</i> < 0.001.
†<i>p</i> < 0.01.
contrast, the $h'$-curve in the presence of quinidine had a slope factor of 15.9 and a midpoint of $-126$ mV. Since in experiments where the $h$-curves exhibited little (<3 mV) drift over the time course of the experimental determinations the $h'$-curve was still shifted by $-26$ mV and its slope factor more than doubled, these observations cannot result from deterioration of the experimental preparation. As in other experiments, the $h'$-curve attained a plateau in the range of $-160$ to $-200$ mV. At the higher dose (50 μM quinidine) the plateau occurred at a smaller amplitude than in the experiments in 20 μM quinidine (Figure 5A).

Thus, hyperpolarization augmented the availability of sodium channels for use-dependent unblocking until a saturating plateau was reached between $-160$ and $-200$ mV. However, compared with the availability of control channels, the availability curve for unblocking was less steep and was shifted toward more negative potentials. As a result, over the potential range of $-120$ to $-160$ mV, where the availability curve of drug-free channels had reached a maximum, the curve representing the availability of sodium channels for unblocking was still increasing.

**Time Dependence of the Fast Recovery Process**

At $-160$ mV (Figure 4) the availability of sodium channels for fast unblocking was quite pronounced in less than 20 msec and did not increase much by 40 or 100 msec. Thus, at $-160$ mV it developed so fast that it approached steady state in less than 20 msec. In 14 experiments recovery intervals as short as 10 msec were used, but again the availability of sodium channels for unblocking had already reached a steady state. At more positive potentials, the amount of use-dependent unblocking was too small, so that even if the kinetics were slower, it would not have been practical to determine them. Consequently, development of availability of sodium channels for unblocking was too fast or too small to be resolved.

If the availability of sodium channels for unblocking is voltage dependent, as described in Figure 5, then after a strong hyperpolarization that fully establishes the availability for unblocking, a subthreshold depolarization preceding the test pulse should reduce the availability for unblocking. In Figure 6, recovery at $-160$, $-140$, and $-120$ mV was determined in the usual way as in Figure 3. In addition, after a 1-second recovery at $-160$ mV, the cell was clamped to $-120$ or $-140$ mV for various times before application of the test pulse (see Figure 6, inset). As the time at less negative potentials was increased, the apparent extra recovery resulting from the hyperpolarization to $-160$ mV declined (filled symbols), until virtually all extra availability of sodium channels for unblocking was lost. The time constant for removal of the availability of sodium channels for unblocking was $191\pm46$ msec at $-120$ mV ($n=14$), much slower than that for removal of inactivation under control conditions ($27\pm8$ msec).

**Recovery From Block During Inactivation**

Little or no block develops during a single long depolarization, but it is not known whether established block is maintained during prolonged inactivation. Therefore, after the block-induction train a long depolarizing clamp (60-second) was imposed to voltages between $-80$ and 0 mV, where all sodium channels are inactivated (see Figure 5). Changes in amplitude of sodium current before and after the 60-second inactivating pulse were compared by application of a test pulse after a 500-msec pulse to $-120$ mV (for removal of inactivation in drug-free channels). In the absence of quinidine, the depolarizing 60-second pulses always reduced the sodium current in a voltage-dependent manner (Table 2), presumably resulting from the development of slow inactivation.

In contrast, in the presence of quinidine (although the currents were smaller than in control), the sodium current always increased after the 60-second depolarizing pulse, indicating that unblocking from quinidine did occur. To the extent that slow inactivation reduced the sodium current, the unblocking of quinidine would even be underestimated. As the 60-second clamp was made more positive, the increase in sodium current declined. This decline may result from development of slow inactivation and block of slowly inactivated sodium channels.

**Discussion**

**Use-Dependent Block**

The present results clearly demonstrate that under appropriate conditions block of sodium channels in
cardiocytes is use dependent as in multicellular preparations.2,3 Our results differ from those of Lee et al.25 who did not observe use-dependent blocking by quinidine in rat myocytes. Although it is possible that the different results stem from a species difference, we consider another explanation more likely for two reasons: 1) In a few experiments done on neonatal rat myocytes we did observe use-dependent blocking (unpublished observations); and 2) our results indicated that the pulse patterns used by Lee et al.25 (applied from a holding potential where h is not maximal) would maintain use-dependent block at very high levels. At potentials positive to −100 mV the fast recovery process is virtually unavailable (Figure 5), and the slow recovery process is so slow (many seconds) that it would take a very long time before significant recovery from block would develop. Therefore, the block by quinidine would remain substantial, and, consequently, little further use-dependent blocking could develop. A similar explanation was provided for the lack of use-dependent blocking by lidocaine.26

The use-dependent block that developed was mostly associated with activation, and not with inactivation, since long depolarizations were not very effective in promotion of block.18 More importantly, previously established use-dependent blocking declined during prolonged depolarizations to potentials above −80 mV (Table 2), where at 17°C sodium channels are inactivated (Figure 5). Thus, frequent activations established a level of block by quinidine that inactivated channels could not maintain.

Recovery From Block

Recovery from block by quinidine consisted of two major components: 1) a fast process and 2) a slow process. The slow process had a time constant that was much longer than reported previously,3,9,10,27,28 but this was probably related to the colder temperature (about 17°C) of the present experiments. Recovery time constants for other drugs are also greatly prolonged at colder temperatures.26,29 Furthermore, in a few experiments when we warmed the preparation to 25°C, recovery from block became much faster.11

As previously described by Weld et al.10 the slow recovery from quinidine exhibited only a moderate voltage dependence. Over the voltage range of −120 to −160 mV, the time constant shortened from 90 to 34 seconds. Thus, contrary to the interpretation of Hondeghem and Katzung,3 diastolic recovery from quinidine block remained slow, even upon strong hyperpolarization (Figure 3).

Use-Dependent Unblocking

Nevertheless, our results (Figure 4) do confirm the previous observation by Hondeghem and Katzung9 that use-dependent blocking is greatly reduced by hyperpolarization. Block associated with each activation combined with slow recovery from block is incompatible with lack of use-dependent block, unless there also exists a fast pathway for unblocking. In the present experiments we demonstrated the existence of a fast component for unblocking: Even after a block-induction train that blocked 97% of the sodium channels, the sodium current recovered to 27% of control in less than 20 msec (Figure 4). This fast unblocking is associated with activation since 1) prolonged hyperpolarization to −160 mV only slowly augments the sodium current, and 2) most importantly, application of several activations caused far more fast unblocking, which could not be accounted for by the slow recovery process. For example, application of five activations from −160 mV increased the sodium current by an additional 35% within a 1-second time span, while it would have required 15 seconds for development of the same level of recovery in the absence of activations (Figure 4). Thus, after establishing block, use of the channels can promote recovery from block (use-dependent unblocking).17,30,31

Like use-dependent blocking, use-dependent unblocking is also time and voltage dependent. Under the conditions of our experiments, use-dependent unblocking increased with hyperpolarization and reached a plateau near −160 mV (i.e., over a potential range much more negative than for removal of inactivation in drug-free channels). Moreover, this fast unblocking process occurred in less than 10 msec (the shortest recovery time studied in the present experiments) over the range of −140 to −200 mV.

The curves that relate the current on the first activation to the preceding recovery voltage are in effect isochronal (1-second) recovery curves. However, the recovery kinetics (Figure 4) indicate that the fast process (τ<100 msec) should have reached steady state, while the slow process contributes only minimally to this curve (<3% for τreco=34 seconds, =1% for τreco=90 seconds). Thus, in Figure 5A the h' value of 0.28 at −160 mV would become 0.25−0.26, and the value of 0.12 at −120 mV would become 0.11. This is an almost proportional bias,

| Table 2. Ratio of Sodium Current With 60-Second Inactivation to Sodium Current Without 60-Second Inactivation |
| --- | --- | --- | --- |
| E<sub>m</sub> (mV) | n | Control | Quinidine | p |
| −80 | 5 | 0.83±0.03 | 4 | 1.76±0.20 | <0.001 |
| −60 | 5 | 0.73±0.05 | 4 | 1.63±0.17 | <0.001 |
| −40 | 5 | 0.60±0.09 | 3 | 1.34±0.27 | <0.001 |
| −20 | 4 | 0.55±0.08 | 2 | 1.34±0.35 | <0.005 |
| 0 | 3 | 0.53±0.09 | 2 | 1.34±0.37 | <0.01 |

For value with 60-second inactivation, block-induction train was followed by a 60-second inactivation clamp to E<sub>m</sub>, then a 500-msec period to −120 mV, then test pulse; for value without 60-second inactivation, block-induction train was followed by a 500-msec period to −120 mV, then test pulse. Mean±SD of ratio of sodium currents is given for control and in presence of 20 μM quinidine. p values represent statistical significance of differences between means as obtained from an unpaired t test. E<sub>m</sub> membrane potential during 60-second clamp; n, number of experiments.
which would have only minimal impact on the slope of the curve and on its midpoint.

On the other hand, one can argue that the observed voltage dependence \( h' \) is contaminated with the voltage dependence of the drug-free channels \( h \): \( h' = h \times f(V) \) where \( f(V) \) represents the true availability of drug-associated channels. However, our experiments were designed to obtain most information over the voltage range where \( h = 1 \), and thus \( h' \) should be a reasonable measure, unless the process of use-dependent unblocking itself is nonlinear.

**Activation Unblock Only Available to Channels in the RD State**

The above use-dependent unblocking induced by hyperpolarization was contingent upon activation that followed the hyperpolarization closely in time.\(^{33}\) Indeed, during a subthreshold depolarization to \(-120\) mV availability of sodium channels for unblocking dissipated with a time constant of \( 191 \pm 46\) msec (Figure 6). Thus, hyperpolarization of drug-associated channels develops and depolarization dissipates a state from which fast recovery from block can occur. By analogy to the regular drug-free sodium channel, we therefore consider this difference in availability to represent the difference between inactivated and rested drug-associated channels. The availability of sodium channels for this fast unblocking process increased over a broader voltage range (\(-100\) to \(-160\) mV) and had a more negative midpoint (around \(-130\) mV) than did the availability for activation of drug-free sodium channels. In modulated receptor terminology (Figure 1; see also below) the hyperpolarization to \(-160\) mV brings most channels from ID (at the end of the induction train) toward RD, while only a fraction moves from ID\( \rightarrow \)RD at \(-120\) mV; during the subthreshold step from \(-160\) to \(-120\) mV, most channels move back from RD\( \rightarrow \)ID, thus dissipating the initially available unblocking capability.

**Recovery Kinetics and the Tertiary Nature of Quinidine**

Slow recovery from quinidine-induced use-dependent \( V_{\text{max}} \) block has also been shown to be pH dependent by Grant et al.\(^{27}\) Extracellular acidification slowed the rate of recovery, suggesting that the charged moiety of the drug (which increases with lower pH) could not readily escape from closed channels. This was further formalized by Starmer and Courtney,\(^{34}\) who viewed the recovery from block in the case of a tertiary compound as a pathway with two reactions in series: 1) deprotonation followed by 2) unblocking of the neutral drug-associated state. The slow time constant of recovery in their model is

\[
\tau_{\text{recov}} = l_d^{-1} (1 + 10^{(pK_a - pH)}) + l_n^{-1}
\]

where \( l_d \) is the deprotonation rate constant and \( l_n \) is the rate constant for neutral drug dissociation from the channel. Upon acidification this formulation predicts a slower \( \tau_{\text{recov}} \) as was shown experimentally for quinidine.\(^{27}\) Our experiments were performed at constant pH, but as protonation/deprotonation reactions involve the transfer of a proton through (part of) the membrane field, a deprotonation pathway is expected to be voltage dependent. If deprotonation were to occur from a generic drug-associated state (non-RD, non-ID), then we would expect \( \tau_{\text{recov}} \) to increase with hyperpolarization, as the increased driving force for protons (from the extracellular phase into the channel) would lower pH in the channel. For every 60-mV increase in driving force, the pH would change with 1 unit. Thus, for a 40-mV hyperpolarization (\(-160\) vs. \(-120\) mV) a 4.6-fold increase in \( H^+ \) concentration or a 0.66 pH drop would be expected. If not all of the membrane field is seen by the proton, which is presumably the case, then the drop would be less. With a \( pK_a = 8.6 \) we only have 5.3% of quinidine in the neutral form at pH 7.35, and at pH 6.69 (7.35–0.66) only 1.2% remains uncharged. Thus, hyperpolarization-induced protonation is expected to slow recovery from the generic drug-associated channel, adding to trapping effect of hyperpolarization on a charged drug\(^{35}\): both gating and hyperpolarization induced protonation act in the same direction. The results presented in this paper, however, show that although recovery was slow at all potentials, it did speed up with time constants increasing from 90 seconds at \(-120\) mV to 34 seconds at \(-160\) mV, contrary to the expected slowing.

The reverse question is whether a hyperpolarization-mediated decrease in channel pH and the resulting increased concentration of charged quinidine could explain the difference in use-dependent unblocking when stepping from \(-160\) mV compared with \(-120\) mV. By use of the above estimates, the fraction of charged quinidine would increase from 95% to 99% as the membrane is hyperpolarized from \(-120\) to \(-160\) mV. Thus, if use-dependent unblocking depends primarily on the initial fraction of charged quinidine-bound channels, only a 4% increase is expected. However, Figure 5A shows that the current during a test pulse increased from 12% to 28% when the recovery potential was changed from \(-120\) to \(-160\) mV, which represents a 2.33-fold increase. It is difficult to see how a 4% increase in charged quinidine would induce a 133% change in current upon depolarization.

**Modulated Receptor Hypothesis**

We interpret these observations as follows: In the presence of quinidine (20 \( \mu M \)) the block-induction train can block nearly all sodium channels, so that during the last depolarization of the block-induction train most of the channels occupy the ID state (Figure 1). As for drug-free channels, hyperpolarization removes inactivation in drug-associated channels (ID\( \rightarrow \)RD, Figure 1), but the voltage dependence for this removal of inactivation is shifted to more negative membrane potentials\(^1\) and appears to have a shallower slope.
Similar to drug-free channels, subthreshold depolarization of rested drug-associated channels results primarily in inactivation without channel opening (RD\(\Rightarrow\)ID), while suprathreshold depolarization induces transient activation of drug-associated channels (i.e., RD\(\Rightarrow\)AD\(\Rightarrow\)ID). Whenever the occupancy of the AD state exceeds the A\(\Leftrightarrow\)AD equilibrium, a fraction of the drug-associated channels unblocks quickly via the AD\(\Rightarrow\)A pathway.

The present results clearly indicate that availability of sodium channels for the fast unblocking process (RD state) is shifted to more negative potentials when compared with availability for activation of drug-free channels (R state). However, the shift may not be a simple parallel one as originally assumed,4 as the voltage dependence of the availability for fast unblocking (\(h'\)-curve) appears significantly less steep than the voltage dependence of the availability for activation in drug-free channels (\(h\)-curve). Thus, if this is a common feature of antiarrhythmic agents, future mathematical formulations of the modulated receptor hypothesis will have to simulate a shallower voltage dependence of inactivation of drug-associated channels.

Preliminary results from simulations with the modulated receptor model 4,9 with a sodium channel formulation appropriate for the lower temperature indicate that once the dissociation rate constant \(l\), is reduced three orders of magnitude, most of our results are fairly well simulated, although a small inflection remains in the simulated \(h'\)-curves (Figure 7). Use-dependent unblocking is best reproduced with a voltage term incorporated in the activated state rate constants to reflect the field effect on the charged drug.17,32 Although more extensive modeling and global fitting are required to obtain more reliable estimates of these rate constants, the concordance between the simulation and the experimental results is striking.

**AD Channels Are Blocked**

The notion that drug-associated channels are “blocked” (i.e., unable to conduct sodium even upon activation) is widely accepted. Nevertheless, there is an alternative explanation: Due to the voltage shift, drug-associated channels could remain inactivated (ID state), and an apparent block would result from “drug-induced inactivation.” The ability for activation of blocked channels increases as the membrane is hyperpolarized (Figure 5), but appears to reach a maximum around \(-160\) mV. This presumably indicates that all channels in the ID state have been translocated to the RD state, so that further hyperpolarization (\(-160\) to \(-200\) mV) fails to increase the already maximal occupancy of the RD state any further. Even after a block-induction train, when nearly all channels are blocked and then transferred to the RD state by strong hyperpolarization, the sodium current of the first depolarization is still reduced. Thus, only a fraction of the drug-associated channels unblocks during each activation, or drug-associated channels do conduct but to a lesser extent.

Since repetitive activations induce cumulative unblocking that occurs much faster than for the slow recovery process (Figure 4), each activation must induce a relief of block via the pathway RD\(\Rightarrow\)AD\(\Rightarrow\)A. We cannot definitely rule out the hypothesis that quinidine-associated channels conduct, but if they do, their conductance must be greatly reduced. We can estimate an upper limit for AD conductance as follows: From Figure 4 the fraction of RD channels has almost saturated at \(-160\) mV. Assuming that all these channels cycle through AD upon the depolarization after full block has been established, the upper limit for AD conductance becomes 28% of control (the amplitude of the \(h'\)-curve is 0.28). However, the steady-state level and the kinetics of use-dependent unblocking (Figure 4) also have to be satisfied, which brings the AD conductance estimate down to less than 10%. A definitive answer to this question will require detailed experiments at the single-channel level. Preliminary results indicate that at the single-channel level, the single-channel conductance remains unchanged, but the channel kinetics are modified (reduction of opening probability).36

**Restored Channels Are Not “Guarded”**

It has been suggested that when the sodium channel is rested, its receptor site is guarded32 or that the rested drug-associated state (RD state in Figure 1) may not exist.34 Our results clearly demonstrated that with increasing hyperpolarization drug-associated channels accumulate in a state from which activation-dependent unblocking and activation can occur. Since only rested channels can activate,6 these drug-associated channels are in the rested state (RD in Figure 1), and rested channels are not guarded from drug occupancy.

According to the modulated receptor hypothesis, once all inactivation is removed from blocked channels by strong hyperpolarizations, that is, all ID channels become RD channels (Figure 1), then all channels become available for activation, and additional hyperpolarization is no longer expected to further enhance use-dependent unblocking. From Figure 5 it is clear that hyperpolarization beyond \(-160\) mV indeed elicits no extra use-dependent unblocking. In terms of the guarded receptor hypothesis, such behavior would require that the voltage-dependent rate constants become independent of voltage. Actually, the guarded receptor hypothesis explicitly suggests that for cationic drugs, recovery from block should continue for potentials beyond the point where \(h'\)=1.32 In contrast, a 1-second recovery period yielded approximately the same amount of recovery from block at \(-160\) and \(-200\) mV (Figures 5A and 5B).

The guarded receptor hypothesis claims that drug occupancy does not alter the time and voltage dependence of channel gating. The present results unequivocally demonstrated that inactivation of drug-associated channels is shifted to more negative potentials and that the slope of the \(h'\)-curve appears
less steep than for the $h$-curve. The guarded receptor can also stimulate availability curves that are shifted to more negative potentials, but contrary to the modulated receptor hypothesis, these curves never exhibit much increase in availability over potential ranges where $h=1$. Furthermore, the slope of the guarded receptor $h'$-curve is steeper than that of control, because there is no true alteration of the voltage dependence of inactivation. The present results confirmed that the availability can increase

**FIGURE 7.** Modulated receptor simulation. Top Panel: Use-dependent unblocking as a function of recovery interval at $-120$ and $-160$ mV. Pulse protocol and symbols as in Figure 4. Open symbols represent first sodium current ($I_{Na}$) after a recovery interval; closed symbols indicate fifth $I_{Na}$ from 5-Hz train started at that recovery interval. As in Figure 4, a marked difference in amount of use-dependent unblocking (during this short 5-Hz train) is obvious, depending on recovery potential. At $-120$ mV hardly any use-dependent unblocking occurs at short recovery intervals, and use-dependent block is noted at later recovery intervals. At $-160$ mV a substantial amount of use-dependent unblocking is evident at short recovery intervals, and as slow recovery process proceeds, less use-dependent unblocking is observed at larger recovery intervals until cross-over point occurs at 50 seconds, where steady-state level of block is 80%; beyond this recovery time use-dependent block develops instead. Middle Panel: Voltage dependence of $I_{Na}$ availability. Symbols indicate individual points from simulation; smooth lines represent a Boltzmann fit through these points. Pulse protocols are shown in inset. Top tracing represents h-curve in control conditions ($k=5.92$ mV, $E_{1/2}=-94$ mV). Bottom tracing shows h'-curve after block induction with quinidine, with an apparently reduced slope and shifted midpoint ($k=16.2$ mV, $E_{1/2}=-115$ mV). Middle tracing shows a substantially unblocked h-curve in presence of quinidine (obtained after an unblocking train of 11 pulses from $-160$ to $-20$ mV, as in Figure 5A) with slope and midpoint close to control values ($k=6.5$ mV, $E_{1/2}=-95$ mV). Bottom Panel: Reduciton of availability for use-dependent unblocking. Symbols and pulse protocols identical to those in Figure 6. Established amount of availability for use-dependent unblocking after 1-second recovery at $-160$ mV declined with depolarization to $-140$ or $-120$ mV. Comparison with Figure 6 shows that modulated receptor model accurately simulates this paradoxical finding. Modeling parameters for all panels: association rate constants (M$^{-1}$msec$^{-1}$) $k_{a1}=k_{a2}=0$, $k_{a3}=2,000$ and 1,150 at $+30$ mV and $-20$ mV, respectively; dissociation rate constants (msec$^{-1}$)$k_{d1}=0.00001$, $k_{d2}=0.00003$, $k_{d3}=0.075$ and 0.1275 at $+30$ mV and $-20$ mV, respectively; quinidine concentration 20 μM; voltage shift $ΔV=40$ mV. Simulations were performed with methods of Hondeghem and Katzung with the following modifications for the α and β equations for the sodium current at 17°C:

$$α_{a}(E)=−0.05×(E+47)/[exp (−0.1×(E+47))−1]$$

$$β_{a}(E)=2×exp (−0.056×(E+72))$$

$$α_{b}(E)=0.0025×exp (−0.09×(E+90))$$

$$β_{b}(E)=0.33[exp (−0.08×(E+38))]+1]$$

$$α_{s}(E)=0.0025×exp (−0.09×(E+90+ΔV))$$

$$β_{s}(E)=0.33[exp (−0.08×(E+38+ΔV))]+1]$$
over the range of −120 to −160 mV, where the control availability has reached a steady state, and that inactivation has a different voltage dependence in drug-associated channels: shifted to more negative potentials and having an apparently shallower slope.

The guarded receptor hypothesis explicitly states that the receptor has a constant affinity for the drug,22 whereas the modulated receptor hypothesis allows a characteristic (possibly, but not necessarily different) affinity for each state. If drug association does not alter gating and the affinity for quinidine remains constant, then according to the guarded receptor hypothesis activation-induced block should persist during a maintained depolarization. In contrast, our results clearly showed that the block established by the induction train declines during maintained inactivation (Table 2).

Finally, according to the guarded receptor hypothesis, recovery from block is voltage dependent because of voltage-dependent rate constants. Thus, after block has been induced, recovery at different potentials should proceed according to the rate constants characteristic for that potential. Specifically, when during the recovery period the potential is stepped from −160 mV to −120 mV (see Figure 6), then recovery should initially occur with a rate constant characteristic of −160 mV. Upon depolarization to −120 mV, the recovery should monotonically continue, but with a slower rate constant characteristic for the −120-mV holding potential. Clearly, the experimental results indicated that this is not the case: After the depolarization to −120 mV, recovery is not only slower, but the block appears paradoxically to increase (filled symbols in Figure 6) before declining again with the rate constant characteristic for the −120-mV holding potential. This result is easily accounted for in terms of the modulated receptor hypothesis. During the −160-mV holding period all drug-associated channels become available for unblock (RD), and the slow unblocking proceeds with a time constant characteristic for the −160-mV holding potential. Upon depolarization the availability for unblocking declines (RD⇒ID) with a time constant of 191±46 msec, while the slow unblocking proceeds with a time constant characteristic of the −120-mV holding potential. Hence, recovery appears as the sum of a quickly declining and a slowly increasing exponential and, thus, has a biphasic appearance. Indeed, the availability for use-dependent unblocking nearly fully dissipates in about 1 second, whereas after recovery proceeds with a rate constant characteristic for the −120-mV holding potential. Actually, the recovery process nearly completely "forgets" that there was a 1-second recovery period at −160 mV, because the difference in the slow recovery processes at −120 and −160 mV was too small in 1 second, and the transient availability for fast unblocking was not consumed. Thus, the paradoxical decline of the sodium current is not caused by block development, but by loss of availability for unblocking during the test pulse.

Because of the above reasons, we concluded that the guarded receptor hypothesis cannot satisfactorily account for our results, but that our results can be explained in terms of the modulated receptor hypothesis.

Clinical Implications

It should be stressed that the quinidine concentrations and the experimental conditions (isolated guinea pig ventricular myocytes, cold temperature, reduced sodium concentration, and ion substitution) used in the present study are very different from typical clinical conditions. Indeed, reduced temperature can considerably alter the effect of antiarrhythmic agents.11,37 Nevertheless, we postulated that the general principles observed should also hold under clinical conditions. It has been shown by numerous laboratories that antiarrhythmic agents selectively depress tissue that is depolarized or that is exposed to tachycardias.38 From the present results, it is clear that for drugs that have long time constants of recovery, the primary route for unblocking is associated with activation. The amount of use-dependent unblocking is greater the more negative the membrane potential. Thus, although quinidine's slow recovery from block is not highly voltage dependent10 and, hence, cannot quantitatively account for the reduced effect upon hyperpolarization,9 its fast recovery from block is substantially voltage dependent. Therefore, the use-dependent block by quinidine is expected to be augmented by depolarization and to be reduced by hyperpolarization. A similar explanation has recently been invoked for the use-dependent unblocking of penticaine, under more physiological conditions.31

At 17°C it was not possible to study the kinetics of the development of the availability of sodium channels for fast unblocking, because they were either too fast or too small in amplitude. However, using maximum upstroke velocity at 37°C, we have observed that the rate of development of availability of sodium channels for fast unblocking is highly voltage dependent and that at −85 mV there is much less use-dependent unblocking per pulse at 3 Hz than at 1 Hz.39 Thus, the rate of repriming of the RD state may be crucially important for the effect of drugs in tachycardias and will need to be fully characterized.

In conclusion, we have shown that quinidine-associated channels also have three primary voltage-dependent states: 1) The RD state is most prevalent at hyperpolarized potentials and is a state from which channels can be activated by depolarization; 2) the AD state can be transiently occupied upon suprathreshold depolarization; and 3) the ID state is most prevalent at depolarized potentials, and channels in this state cannot be activated unless inactivation is first removed. Thus, drug-associated channels have a voltage-dependent behavior that is similar to that of drug-free channels. However, their voltage dependence is shifted to more negative potentials and appears to be a less steep function of membrane
potential. In addition, quinidine-associated channels do not conduct sodium upon activation (or at the very least have a greatly reduced sodium conductance). Sodium channels in the rested and inactivated states appear to have a relatively low affinity for quinidine. Block is primarily associated with activation of the channels. Moreover, interactions with the activated state occur very fast (in the microsecond-to-milli-second range) compared with the much slower interactions with rested and inactivated channels (tens of seconds). Our findings appear compatible with the modulated receptor hypothesis but are incompatible with the guarded receptor hypothesis since the affinity of the sodium channel receptor for quinidine is not fixed, the gating of drug-associated channels has altered time and voltage dependence, recovery from block does not necessarily occur monotonically, and rested channels are not “guarded” from occupation by quinidine.

Addendum: With respect to the above discussion of pH-dependent effects, Courtney40 presented some new results while this paper was under review: that the pH modulation of recovery from block by lidocaine requires that the voltage dependence of the drug-associated channels be strongly modified. This finding, like ours, is incompatible with the guarded receptor principles but is compatible with the modulated receptor.

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