Mechanisms of Quinidine-Induced Depression of Maximum Upstroke Velocity in Ovine Cardiac Purkinje Fibers

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Summary. A major advance in understanding how quinidine depresses maximum upstroke velocity ($V_{\text{max}}$) is the Hondeghem-Katzung mathematical model which incorporates voltage-independent rate constants for binding to and unbinding from resting, open, and inactive Na channels, and a voltage shift of $-40$ mV for the Hodgkin-Huxley $h$-kinetics of quinidine-associated Na channels. Using a double microelectrode voltage clamp technique to control transmembrane voltage and apply conditioning pulses, we found that quinidine blockade increased as transmembrane voltage became more positive in the range $-60$ to $+40$ mV, and that the rate of quinidine dissociation increased as transmembrane voltage became more negative in the range $-60$ to $-140$ mV. The relationship of $V_{\text{max}}$ to transmembrane voltage obtained at drive cycles from 500 msec to 20 seconds conformed to the model modified to include voltage-dependent rate constants without the postulated $-40$-mV shift for quinidine-associated channels. Thus binding of quinidine to inactive Na channels and unbinding from resting channels are both voltage-dependent and can explain frequency and voltage-dependent actions of quinidine on $V_{\text{max}}$ without any voltage shift for quinidine-associated channels. (Circ Res 50: 369-376, 1982)

A characteristic common to most antiarrhythmic drugs is depression of phase 0 of the cardiac action potential. Depression of $V_{\text{max}}$ by quinidine has been found to be dependent upon both frequency of stimulation and transmembrane voltage (Weidmann, 1955b; Johnson and McKinnon, 1957; Heistracher, 1971). A major advance in our understanding of how quinidine acts to depress $V_{\text{max}}$ has been the "modulated receptor hypothesis" model proposed by Hondeghem and Katzung (1977a, 1977b, 1980; see also Hille, 1977; Courtney, 1980) (Fig. 1). This model explains depression of phase 0 upstroke velocity in terms of binding and unbinding of quinidine to the membrane sodium channel, thereby providing insight into the mechanisms of antiarrhythmic action. The Hondeghem-Katzung rate constants for binding and unbinding of quinidine from the sodium channel are not directly voltage-dependent but, instead, are dependent on the configuration of the sodium channel (modulated receptor hypothesis). Transmembrane voltage changes affect quinidine binding by changing the sodium channel among resting, open, and inactive configurations.

Experiments in nerve show additional local anesthetic depression of sodium current during conditioning pulses positive to voltages at which all sodium channels are not directly voltage-dependent but, instead, are dependent on the configuration of the sodium channel (modulated receptor hypothesis). Transmembrane voltage changes affect quinidine binding by changing the sodium channel among resting, open, and inactive configurations.

Methods

Ovine hearts were obtained at a slaughterhouse and transported to the laboratory in an insulated jug containing iced Tyrode's solution equilibrated with 95% O2-5% CO2. Excised 2-mm fiber segments were stored in Tyrode's solution at 20-24°C until studied. The composition of the Tyrode's solution in mmol/liter was: NaCl, 137; NaHCO3, 12; NaH2PO4, 1.8; dextrose, 5.5; CaCl2, 2.4; MgCl2, 0.5; and KC1, 4.0. Quinidine sulfate (Aldrich) was added to the test solution to provide a final concentration of 10 mg/liter (3 X 10-5 M) and express binding rate constants as $k_1(Q)$ and $k_u(Q)$, where units are sec-1.

* In the model (Fig. 1), $k_1$ and $k_u$ are in sec-1, and $l_1$ and $l_u$ are in sec. We used a quinidine concentration of 10 mg/liter (3 X 10-5 M) and express binding rate constants as $k_1(Q)$ and $k_u(Q)$, where units are sec-1.
FIGURE 1. The Hondeghem-Katzung model for the mechanisms of depression of upstroke velocity by quinidine (modified from Hondeghem and Katzung, 1980; Courtney, 1980; Hille, 1977).Membrane Na channels are shown as closed resting (R), active or open (A), and closed inactive (I). Quinidine-associated blocked channels are shown by R', A' and I'. Rate constants for association of quinidine with each channel state are indicated by subscripted k's, while rate constants for dissociation of quinidine from channels are shown in subscripted l's. Recovery of sodium channels from the I to R state occurs with the voltage-dependent Hodgkin-Huxley rate constant $a_h(V)$, whereas the voltage rate constant for recovery of quinidine-blocked channels, $a_h(V + \Delta V)$, has its voltage dependence shifted by $\Delta V$ (about $-40 \, \text{mV}$). The shaded areas of the model are tested by the experiments of the present investigation.

maintained at 36°C. Glass capillary microelectrodes for recording transmembrane voltage were filled with 3 M KCl. Microelectrodes for passing current were filled with 2 M potassium citrate. The voltage clamp circuit, display and photographic apparatus, bath, heating, and circulating equipment have been described previously (Weld and Bigger, 1975, 1976).

To study quinidine association with closed inactive sodium channels, we used a voltage clamp to apply depolarized conditioning pulses more positive than $-40 \, \text{mV}$ (where sodium channels are in the closed and inactive state), and varied either conditioning voltage or conditioning pulse interval (see Figs. 2 and 3). At the end of the conditioning pulse, the fiber was clamped back to the holding voltage of $-80 \, \text{mV}$ for 200 msec, long enough to enable quinidine-free sodium channels to reactivate fully, but short enough so that negligible dissociation of quinidine from blocked channels occurred (Hondeghem and Katzung, 1977a, 1977b, 1980; vide infra). At the end of the 200-msec holding interval, we opened the voltage clamp with an electronic relay, immediately stimulated the membrane with a 0.1-msec rectangular pulse via external electrodes (within 1 msec after disconnecting the voltage clamp), and measured $V_{\text{max}}$ of the action potential upstroke.

clamped the fiber at a desired holding voltage. After 20 seconds, we then disconnected the voltage clamp for 20 msec with an electronic relay, immediately stimulated the membrane with a 0.1-msec rectangular pulse via external electrodes (within 1 msec after disconnecting the voltage clamp), and measured $V_{\text{max}}$ of the action potential upstroke.

FIGURE 2. Blockade of inactive sodium channels by quinidine. Experimental technique shown in inset. At the end of each conditioning pulse ($V_c$), the fiber is repolarized to the holding voltage ($V_h$) for 200 msec and then an action potential upstroke ($V_{\text{max}}$) is elicited (arrow) and maximum upstroke velocity, $V_{\text{max, peak}}$, is measured. $V_{\text{max}}$ decreases in an exponential fashion as conditioning pulse interval is increased. At the conditioning pulse voltage of $+13 \, \text{mV}$, membrane sodium channels are in the closed inactive configuration, so the progressive fall in $V_{\text{max}}$ with longer conditioning pulse intervals reflects progressive blockade of inactive sodium channels by quinidine.

FIGURE 3. Voltage dependence of blockade of inactive sodium channels by quinidine. Experimental technique shown in inset. Following a 2-second conditioning pulse, an action potential is elicited (arrow) and $V_{\text{max}}$ is measured. As the conditioning pulse voltage becomes more positive, $V_{\text{max}}$ shows more depression, reflecting enhanced blockade of inactive channels by quinidine.
obtained. We then changed the holding clamp voltage and repeated the procedure. This was repeated for voltages from −60 to −140 mV to obtain the steady state Vmax-activation voltage relationship. To obtain this relationship for 500-msec cycles, we similarly measured Vmax of action potential upstrokes elicited every 500 msec instead of every 20 seconds. After changing holding clamp voltage, we did not measure Vmax until Vmax reached its steady state value.

Data were used from experiments where impalements were maintained throughout control, quinidine, and washout. Data are mean ± SD. First order regressions were fit by the method of least squares. Statistical significance of differences between control and quinidine data was determined by the t-test for paired samples (Snedecor and Cochran, 1978).

Results

Effects of Quinidine on the Purkinje Fiber Action Potential

Based on preliminary dose-ranging experiments, we chose to study a quinidine concentration of 10 mg/liter. Depression of Vmax of action potential phase 0 ranges from negligible (when the membrane is hyperpolarized) to marked (when preceded by a depolarized conditioning pulse) at this concentration; thus we could examine drug binding to and unbinding from a majority of the membrane sodium channels (vide infra). During superfusion of 11 Purkinje fiber preparations with quinidine 10 mg/liter, action potentials elicited at a 1000-msec cycle showed depolarization of membrane activation voltage (voltage from which phase 0 begins) from −85.2 ± 6.2 mV to −78.6 ± 7.0 mV; depolarization of maximum diastolic voltage from −86.5 ± 6.4 mV to −79.8 ± 7.7 mV; decrease in Vmax from 589 ± 128 V/sec to 277 ± 82 V/sec; decrease in overshoot from 33.6 ± 7.0 mV to 15.5 ± 6.7 mV; decrease of action potential duration at 50%

![Figure 4](image-url)  Method for determination of Vmax recovery with the voltage clamp. Top trace: transmembrane current (damped). Second trace from top: extracellular zero voltage reference. Third trace: transmembrane voltage. Top three traces are referenced to time scale at top. Bottom trace: first time derivative of voltage at the time of action potential phase 0, inscribed at high oscilloscopic sweep speed from right to left (bottom time scale). The terminal portion of a conditioning pulse at −15 mV is seen at the left side of the photograph; 230 msec later, the voltage clamp is disconnected (small artifact at right edge of V trace) and the fiber stimulated with external electrodes with an 0.1-msec stimulus (spike artifact immediately to the right of Vmax) to generate an action potential upstroke. See text for details of method.

![Figure 5](image-url)  Removal of quinidine blockade of closed resting channels. Experimental technique shown in inset. Following a 2-second +20-mV conditioning pulse, an action potential upstroke is elicited (arrow) at different recovery intervals, and the Vmax is divided by peak Vmax (obtained after 20 seconds or longer at Vh) to give the available fraction of Vmax. The unavailable fraction of Vmax, reflecting quinidine-associated blocked resting channels, declines exponentially during recovery from the conditioning pulse. Note that there is an early very rapid decline of blocked channels similar to control recovery. This rapidly recovering Vmax probably represents membrane channels not associated with quinidine.

![Figure 6](image-url)  Removal of quinidine-blocked Vmax following conditioning pulses of different magnitude. Experimental technique identical to that in Figure 5. Only the slow phase of recovery is shown. When a more depolarized conditioning pulse is used, more Vmax is blocked (i.e., the y-intercept becomes larger at the expense of the rapidly recovering fraction seen in Figure 5) but recovery kinetics are unchanged. Thus the fraction of quinidine-blocked channels can be increased with a more depolarized conditioning pulse, but, upon repolarization to Vh, quinidine dissociates from these channels with the same kinetics, independent of the magnitude of quinidine blockade.
repolarization from 313 ± 88 msec to 166 ± 117 msec (all changes significant at \( P < 0.05 \)), and slowing of
terminal repolarization. There was no significant
change in the magnitude of phase 4 depolarization.

Voltage Dependence of Quinidine Binding to
Inactivated Sodium Channels

To determine blocking of inactive sodium channels
by quinidine, we measured \( V_{\text{max}} \) after a depolarized
conditioning pulse of variable duration. Depression
of \( V_{\text{max}} \) increased in an exponential fashion as the
duration of the conditioning voltage clamp increased
(Fig. 2).

To determine the voltage dependence of the inac-
tivation of \( V_{\text{max}} \) by quinidine during conditioning
pulses, we varied the magnitude of the conditioning
pulse (Fig. 3). There was little voltage-dependent de-
pression of \( V_{\text{max}} \) when conditioning pulses were
more negative than −60 mV. At conditioning pulse
voltages more positive than −60 mV, there was a
progressive increase in the magnitude of \( V_{\text{max}} \) depres-
sion. Derivation of the rate constants for binding with
(\( k_{\text{d}}[Q] \)) and unbinding from (\( l \)) sodium channels from
these data is given in the Discussion. (\( k_{\text{d}}[Q] \) is the
binding rate constant in sec\(^{-1} \) for a quinidine concen-
tration of 10 mg/liter, or \( 3 \times 10^{-5} \) m.)

Voltage Dependence of Quinidine Dissociation
from Resting Sodium Channels

We evaluated recovery from quinidine-induced
\( V_{\text{max}} \) depression by plotting \( V_{\text{max}} \) of action potentials
against the recovery interval following a fixed depo-
larized conditioning pulse. Removal of inactivation is
obtained by plotting against recovery time the differ-
ence between \( V_{\text{max}} \) measured during recovery and peak \( V_{\text{max}} \).
Figure 5 shows removal of inactivation at
−80 mV following a 2-second conditioning pulse at
+20 mV, plotted on a semilogarithmic scale. In all
quinidine experiments, a rapid phase of \( V_{\text{max}} \) recov-
ery, with kinetics comparable to recovery in control
solution, preceded a slow phase of recovery, repre-
senting recovery of \( V_{\text{max}} \) from quinidine depression
(Fig. 5). The fraction of \( V_{\text{max}} \) blocked by quinidine
during the conditioning pulse could be increased by
making the pulse either longer or more depolarized,
but the first order kinetics of the recovery process
following the conditioning pulse were unaltered (Fig.
6). Derivation of the rate constant for dissociation of
quinidine from resting channels (\( l_{R} \)) from these data
is given in the Discussion.

Quinidine-Induced Voltage Shifts

To test the hypothesis that quinidine shifts the
electrical dependence of the magnitude of \( V_{\text{max}} \), we
determined the relationship of \( V_{\text{max}} \) to activation volt-
age. First, we found that clamping for longer than 20
seconds prior to an action potential upstroke during
exposure to quinidine did not result in further recov-
ery of \( V_{\text{max}} \), so we concluded that the steady state
\( V_{\text{max}} \)-activation voltage relationship can be obtained
at a drive cycle of 20 seconds (Fig. 7). In 12 steady
state \( V_{\text{max}} \)-activation voltage experiments, quinidine
minimally shifted the voltage at half-maximal \( V_{\text{max}} \)
(\( V_{\text{d}} \)) from −64.2 ± 7.3 mV to −67.0 ± 7.1 mV (\( P <
0.02 \)) at a 20-second drive cycle. The peak \( V_{\text{max}} \) ob-
tainable was not diminished by quinidine: hyperpo-
larization to −90 mV or more negative at a drive cycle
of 20 seconds restored the peak \( V_{\text{max}} \) in quinidine
(556 ± 219 V/sec) to control values (562 ± 203 V/sec,
difference not significant).

Because the Hondeghem-Katzung model (Fig. 1)
uses rate constants for drug binding which are not
voltage-dependent, the model is obliged to account
for more rapid reactivation of \( V_{\text{max}} \) at more negative voltages by postulating a voltage shift of about −40
mV for the Hodgkin-Huxley \( \alpha_{0} \) of quinidine-associ-
cated channels. In view of our finding that rate con-
stants for quinidine binding and unbinding are de-
pendent upon transmembrane voltage, we wondered
whether the Hondeghem-Katzung mathematical
model actually required the postulated −40 mV volt-
age shift for Hodgkin-Huxley kinetics of drug-asso-
ciated channels. To evaluate whether such a voltage
shift for the Hodgkin-Huxley kinetics of quinidine-
blocked channels actually exists, we tested the Hon-
deghem-Katzung model prediction (which results from
the postulated voltage shift) that the relationship
between $V_{\text{max}}$ and activation voltage exhibits an inflection, or double sigmoid configuration, as drive cycle is shortened. We found that abbreviation of the drive cycle to 500 msec during exposure to quinidine produced a marked shift of the $V_{\text{max}}$-activation voltage relationship downward and to more negative voltages (Fig. 7). We did not observe the predicted inflection, or double sigmoid shape, of the $V_{\text{max}}$-activation voltage relationship in any of 15 experiments, and we were unable to restore peak $V_{\text{max}}$ to control values at a 500-msec cycle even with membrane hyperpolarization to $-140$ mV prior to eliciting an action potential upstroke (Fig. 7).

**Discussion**

Voltage Dependence of Quinidine Binding to Inactivated Sodium Channels

To obtain $k_i[Q]$ and $l_i$, the rate constants for blocking and unblocking inactive sodium channels (Hodgkin-Huxley h gate closed) by quinidine, we used the time course for and the steady state value of depression of $V_{\text{max}}$ by quinidine during sustained depolarized pulses. The time course of blocking the inactivated sodium channel by quinidine in Figure 2 is approximated well by an exponential fit. If $l'$ represents the fraction of inactive sodium channels associated with quinidine, we can express the change in quinidine-associated channels as:

$$\frac{dl'}{dt} = k_i(V)[Q](1 - l') - h(V)l'$$

where $l_0$ is the rate constant for dissociation of quinidine from quinidine-associated inactive channels and $V$ is transmembrane voltage. Prior to a depolarized pulse, we held transmembrane voltage adequately negative to ensure that $l' = 0$ at $t = 0$. Therefore, during a sustained depolarized pulse, the amount of block is given by (cf. Courtney, 1980):

$$l' = l_{\text{ss}}(1 - e^{-k_i(V)[Q]+1-V(1/v)})$$

where $l_{\text{ss}}$ is the steady state fraction of quinidine-associated inactive channels at the test voltage, and:

$$l_{\text{ss}} = \frac{(k_i(V)[Q])}{(k_i(V)[Q] + h(V))}$$

The term $(k_i(V)[Q] + l(V))$ in Equation 2 is the slope of the exponential regression in Figure 2. The value of $l_{\text{ss}}$ in Equation 3 is the difference between $V_{\text{max}}$ at zero time and at "infinite" time (asymptotic value of the exponential regression) in Figure 2. We used Equations 2 and 3 to obtain $k_i[Q]$ and $l_i$ at different test voltages (Fig. 8). Figure 8 also includes the Hondeghem-Katzung model values for $k_i[Q]$ and $l_i$ in order to highlight the differences between the model values and experimental data.

Voltage Dependence of Quinidine Dissociation from Resting Sodium Channels

To obtain $l_R$, the rate constant for unblocking the resting sodium channels (Hodgkin-Huxley h gate open) by quinidine, we used the time course for recovery of $V_{\text{max}}$ from quinidine depression. Recovery, plotted as removal of quinidine block in Figure 5, is approximated well by an exponential process. If $R'$ represents the fraction of resting sodium channels associated with quinidine, we can express the change in quinidine-associated channels as:

$$\frac{dR'}{dt} = k_R(V)[Q](1 - R') - l_R(V)R'$$

where $k_R(V)[Q]$ is the rate constant for blocking of resting channels by quinidine. Since recovery of $V_{\text{max}}$ from quinidine depression proceeds to completion if enough time elapses (except possibly in the narrow voltage range of the steep portion of the $V_{\text{max}}$-activation voltage relationship), $l_R$ must be much larger than $k_R[Q]$, and the last term in Equation 4 becomes negligibly small. The solution to Equation 4 then becomes

$$R' = R'_{\text{cond}}(e^{-lt})$$

where $R'_{\text{cond}}$ is the fraction of quinidine-associated channels after the depolarized conditioning pulse ($y$-
axis intercept for slow recovery of \( V_{\text{max}} \) in Figs. 5 and 6. The rate constant for dissociation of quinidine from quinidine-blocked channels becomes larger as transmembrane voltage becomes more negative. The linear equation which best describes this relationship is shown. For comparison, the Hondeghem-Katzung model value for \( I_R \) (horizontal line) is also shown. Data are from 12 experiments in nine fibers.

**Quinidine-Induced Voltage Shifts**

In the Hondeghem-Katzung mathematical model (1977a, 1977b, 1980), quinidine causes a voltage shift of \(-40\) mV in the voltage dependence of the Hodgkin-Huxley \( \alpha_0 \) rate constant for recovery of quinidine-associated channels from I to R' (Fig. 1), but not for recovery of quinidine-free channels from I to R. The postulated voltage shift predicts that the single sigmoid curve of the \( V_{\text{max}} \)-activation voltage relationship obtained at long drive cycles will develop an inflection (dashed line in Fig. 7) when drive cycle is shortened to 500 msec, and that \( V_{\text{max}} \) will attain its peak value with hyperpolarization to \(-140\) mV. We did not find an inflection (double sigmoid configuration) in the \( V_{\text{max}} \)-activation voltage relationship at drive cycles as short as 500 msec, and \( V_{\text{max}} \) at 500-msec cycles never reached peak \( V_{\text{max}} \) even at \(-140\) mV (e.g., Fig. 7). These findings are not consistent with the \(-40\) mV voltage shift postulated by Hondeghem and Katzung for quinidine-associated channels, but can be explained fully by voltage dependence of the rate constant, \( I_R \), for quinidine unbinding from resting channels.

The \( V_{\text{max}} \)-activation voltage relationship at a 20-second drive cycle (e.g., Fig. 7) showed a mean quinidine-induced shift of \(-2.8\) mV on the voltage axis which could not be decreased by increasing drive cycle beyond 20 seconds. The most likely cause for this small voltage shift is that the association rate constant for quinidine binding to resting channels, \( k_d(Q) \), is significant in the voltage range of the steep portion of the \( V_{\text{max}} \)-activation voltage relationship. Our results predict that \( K_D \), the equilibrium dissociation constant, is voltage-dependent, and the value of \( k_d(Q) \) at any particular voltage will depend on the relative magnitude of \( K_D \) at that voltage compared to the concentration of quinidine used. Therefore, only at less negative voltages (\(-60\) to \(-75\) mV), where the concentration of quinidine we used (10 mg/liter, or 30 \( \mu \)m) approaches the value of \( K_D \) will \( k_d(Q) \) be significant with respect to \( I_R \). Another possible explanation for the small negative shift of the steady state \( V_{\text{max}} \)-activation voltage relationship by quinidine is an “charge shielding” action of quinidine at the cell membrane, affecting all membrane sodium channels regardless of association with quinidine.

**Significance of the Present Experiments**

Implicit in our experiments is the assumption that \( V_{\text{max}} \) is a measure of sodium current and sodium conductance. Although cardiac electrophysiologists have used \( V_{\text{max}} \) as an indirect measure of sodium conductance since Weidmann’s study in 1955, this has been and is an area of considerable controversy. Theoretical arguments based on computer modeling of action potential currents have led to both support (Hondeghem, 1978; Walton and Fozzard, 1979) and opposition (Hunter et al., 1975; Cohen and Strichartz, 1977; Strichartz and Cohen, 1978) to the concept that \( V_{\text{max}} \) is a valid measure of sodium current and sodium conductance. Bean et al. (1981) and Cohen et al. (1981) have suggested that under certain conditions in rabbit Purkinje fibers, \( V_{\text{max}} \) is a non-linear measure of sodium conductance. Preliminary investigations in our own laboratory show that some of the theoretical objections to the use of \( V_{\text{max}} \) as a measure of sodium conductance are not significant limitations under certain experimental conditions (Coromilas et al., 1981). Whereas the validity of \( V_{\text{max}} \) as a linear measure of peak sodium current and conductance must still be considered an unsettled issue, there is little doubt that \( V_{\text{max}} \) is generated by the early inward transient current and provides valuable indirect information about changes in this current.

Chen et al. (1975) and Chen and Gettes (1976) concluded that quinidine did not alter the recovery kinetics of \( V_{\text{max}} \), but that quinidine did depress \( V_{\text{max}} \) in a voltage-dependent manner which was more marked at higher drive rates. Chen and Gettes (1976) felt the most likely explanation for enhanced depression of \( V_{\text{max}} \) at higher drive rates was depression of the ATPase-dependent Na-K pump by quinidine, resulting in a decrease in the transmembrane sodium gradient, which became more marked as the drive rate (and sodium entry into the cell) increased. However, both Hondeghem and Katzung (1977) and we have shown that quinidine does slow recovery kinetics of \( V_{\text{max}} \). Also, we found that the most intense quinidine-induced depression of \( V_{\text{max}} \) occurred at the most positive conditioning voltages, where the driving
force for entry of sodium into the cell is the least. Therefore, it is not necessary to postulate fatigue of the Na-K pump (and intracellular Na accumulation) to account for depression of \( V_{\text{max}} \) by quinidine. Our data on recovery from depression of \( V_{\text{max}} \) by quinidine are most consistent with time- and voltage-dependent unblocking of membrane sodium channels by quinidine.

The rate constants for blocking and unblocking of inactive channels by quinidine in the transmembrane voltage range of the action potential plateau suggests that little binding of quinidine occurs during the plateau. Association of quinidine to membrane sodium channels therefore must occur predominantly during phase 0 (Hondeghem and Katzung, 1980). Our data suggest that antiarrhythmic efficacy (which is associated with depression of \( V_{\text{max}} \)) may be more pronounced as heart rate increases and recovery time decreases. Likewise, a high quinidine concentration may be safe at a slow heart rate but may become toxic at more rapid rates. Quinidine-toxic depression of \( V_{\text{max}} \) should be reversed best by maneuvers to hyperpolarize the cell membrane and to avoid rapid heart rates. Further, although there is only minor association of quinidine with inactive sodium channels during the action potential plateau, abbreviation of the action potential should nonetheless antagonize quinidine-induced depression of \( V_{\text{max}} \) by virtue of the resultant increase in recovery time after repolarization.

The existing Hondeghem-Katzung model (Hondeghem and Katzung, 1980) for depression of \( V_{\text{max}} \) by quinidine utilizes the modulated receptor hypothesis (Hille, 1977; Hondeghem and Katzung, 1977a, 1977b; Fig. 1). According to this model, a sodium channel in any of the resting, open, or inactive states can combine with quinidine and thereby be blocked. Each channel state (resting, open, and inactive) has characteristic rate constants for quinidine binding and unbinding; these rate constants do not vary with transmembrane voltage. The present experiments indicate that binding of quinidine to inactive sodium channels and unbinding of quinidine from resting sodium channels are, in fact, strongly voltage-dependent. Thus, transmembrane voltage modulates either the access of quinidine to its channel-blocking position or else the kinetics of the actual association of quinidine with its channel-blocking site (receptor). Since the degree of openness of inactive channels should decrease with more depolarized voltages (i.e., the steady state value of Hodgkin-Huxley \( m \) should decrease), we doubt that the increase in \( k_d(Q) \) at positive transmembrane voltages reflects enhanced access of quinidine to its binding site through the channels themselves. Instead, we favor either voltage-enhanced association of quinidine with its blocking site, perhaps through a voltage-dependent change in channel geometry at the inner surface of the membrane, or a voltage-enhanced movement of quinidine across non-channel portions of the membrane to the cell interior. In deciding between these two possibilities, the kinetics of quinidine blockage are helpful. Since extracellular quinidine is relatively constant, we would expect a voltage-enhanced movement of quinidine into the cell to exhibit zero-order kinetics. However, the enhancement of block by depolarized voltages exhibits an exponential time course (see Fig. 2). This evidence favors a voltage-modulated association of quinidine with its blocking site.

In summary, the present experiments show that binding of quinidine to inactive channels and unbinding from resting sodium channels are voltage-dependent. Further, our data indicate that there is no significant shift for the Hodgkin-Huxley h-kinetics of quinidine-associated channels. These suggested changes for the Hondeghem-Katzung model result in a more accurate and more powerful model for the interaction of quinidine with the cardiac Purkinje fiber membrane sodium channel.

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