Single inward rectifier potassium channels in guinea pig ventricular myocytes

Effects of quinidine

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ABSTRACT The effects of quinidine on single inward rectifier K channels were investigated in cell-attached patches with 4.5 mM pipette potassium concentrations. Under these conditions, the single-channel slope conductance of the predominant conductance level of the inward rectifier channels was 3.9 ± 0.3 pS at membrane potentials between −75 and −150 mV. Quinidine reversibly decreased the likelihood of channel opening to the main conductance level without reducing the single-channel conductance, and also reduced the probability of channel opening to subconducting levels. Quinidine had no significant effects on the channel open times, and the inhibition of channel opening was only slightly voltage dependent over the range of membrane potentials investigated. Quinidine induced a complete cessation of channel openings for brief periods (up to 2 min), suggesting that quinidine promoted occupancy of a state from which opening was less likely. Occasional long periods (up to an hour) with an absence of channel activity were also observed but quinidine did not appear to promote this behavior. The data suggest that quinidine decreases the ability of the channel to enter both main and subconducting states. By binding to a particular closed conformation of the channel, quinidine could reduce the likelihood of channel opening. The main features of these observations could be accounted for using the three-state kinetic model proposed by Sakmann, B. and G. Trube (1984*b*. *J. Physiol. [Lond.]*. 347:659–683.) with quinidine binding to the middle closed state.

INTRODUCTION

Quinidine, one of the oldest antiarrhythmic agents in widespread usage, is a naturally occurring cinchona alkaloid that suppresses a number of distinct cardiac potassium currents. These include delayed rectifier potassium currents (I_K or I_x; Colatsky, 1982; Hiraoka et al., 1986; Roden et al., 1988; Furukawa et al., 1989), transient outward currents (Imaizumi and Giles, 1987), and inward rectifier (time-independent) K currents (Hiraoka et al., 1986; Salata and Wasserstrom, 1988). However, the mechanisms which underlie block of cardiac potassium channels by quinidine have not been characterized at the single-channel level. Unlike delayed rectifier and transiently opening K channels in cardiac cells, inward rectifier K channel activity can be recorded for long periods in cell attached patches; hence, its single channel properties have been well characterized (Sakman and Trube, 1984*a*; Trube and Hescheler, 1984; Matsuda, 1988). We have investigated the effects of quinidine on single inward rectifier potassium channels in isolated guinea pig ventricular cells.

In many cases ion channels in patches of membrane excised from cells have altered gating behavior, and in particular inward rectifier channel activity sometimes ceases after patch excision (Trube and Hescheler, 1984). We have therefore attempted to preserve the intracellular milieu of the channel by using the cell-attached patch configuration of the patch-clamp method (Hamill et al., 1981). Recently, Salata and Wasserstrom (1988) described suppression of the time-independent potassium current (I_{K1}) in dog ventricular myocytes. Their work showed irreversible reduction of the steady-state outward and inward current (steady-state current above and below E_K). A number of studies have indicated that no detectable outward current flows through single inward rectifier K channels in intact cells (Sakmann and Trube, 1984*a*; Josephson and Brown, 1986; Mazzanti and DeFelice, 1988). In fact, outward currents have only been observed at the single-channel level when the intracellular side of the patch was exposed to artificially low levels of Mg^{++} (Vandenberg, 1987; Matsuda, 1988). Hence, the mechanism responsible for suppression of the outward steady-state current by quinidine remains in question. However, the macroscopic inward current suppressed by quinidine at membrane potentials negative to E_K most likely results from block of inward rectifier K channels; we have attempted to clarify this by investigating the effects of quinidine on single inward rectifier K channel openings at membrane potentials below E_K. Previous studies of single inward rectifier K channels have utilized high extracellular potassium concentrations (10.8–300 mM), presumably to increase the ability to resolve single K channel openings (Sakmann

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and Trube, 1984*b*; Trube and Hescheler, 1984; Matsuda, 1988). However it is known, at least for Na channels, that the concentration of permeant ion can alter the block caused by some drugs (Cahalan and Almers, 1979). It is also known that the conductance and kinetics of the inward rectifier potassium channel are altered by changes in external potassium concentration (Sakmann and Trube, 1984*b*; Cohen et al., 1989); alteration of these channel properties may secondarily affect drug-channel interactions. Therefore, we have studied the effects of quinidine on these single K channels under conditions of normal extracellular potassium concentration.

METHODS

Cell preparation

Acutely isolated guinea pig ventricular myocytes were prepared as previously described (Farmer, 1983; Bennett et al., 1987). Hearts were perfused via the ascending aorta with a minimum essential medium containing collagenase (200 U/ml; Worthington type II; Cooper Biomedical, Malvern, PA). After perfusion for 15 min the ventricles were minced, washed and filtered through 200 μm teflon mesh. The cells were then placed in Medium 199 (Sigma Chemical Co., St. Louis, MO) with 1 mM calcium and stored in an incubator at 37°C until used within 12 h.

Solutions

Ionic currents through other channels and transport systems were suppressed by ionic substitution and blockers. Patch pipettes usually contained (in millimolar): KCl 4.5; *N*-methyl-*d*-glucamine-Cl 150; $MgCl_2$ 2.0; $CaCl_2$ 0.1; $CdCl_2$ 0.1; $LaCl_3$ 0.1; Hepes 20; glucose 12. *N*-methyl-*d*-glucamine was used as an ionic substitute for Na^+. Channel blockers (Cd^{++}, La^{+++}) and a reduced extracellular calcium concentration were used to eliminate calcium-dependent currents. External calcium was lowered to 0.1 mM, but was not eliminated completely, to avoid permeation of Ca channels by other ions (Hess and Tsien, 1984). The bath usually contained (in millimolar): NaCl 145; Hepes 10; $MgCl_2$ 1.0; $CaCl_2$ 1.8; KCl 4.5; glucose 10. These solutions maximized isolation of potassium currents while minimizing contributions from other ionic currents which in turn will improve voltage clamp control and prevent inadvertent uncontrolled changes in membrane potential. Exceptions to these solutions are noted in the figure legends and text. All experiments were performed at 22–23°C.

Patch clamp recording

Patch electrodes were made from capillary tubing (Model 1812; Radnoti Glass Technology, Inc., Monrovia, CA), and after heat polishing had resistances of 9 ± 4 MΩ when filled with the solution listed above. Electrodes were coated near the tip with Sylgard (Compound 184; Dow-Corning Corp., Midland, MI) to reduce capacitance and noise. This allowed resolution of K channel openings at membrane potentials within 15 mV of E_K. To resolve the small unitary currents (0.05 pA) required for this study, it was necessary to obtain seal resistances in excess of 100 GΩ (Hamill et al., 1981). Thus, we report here only the results from patches meeting these criteria (780 ± 510 GΩ; $N = 9$). In most experiments, current records were low pass filtered at 100 Hz (−3dB; four-pole Bessel filter) before sampling at 500 Hz (five times the filter cut-off frequency) by a 12-bit analog-to-digital converter controlled by a PC/AT microcomputer (IBM Instruments, Inc., IBM Corp., Danbury, CT); exceptions are noted in the figure legends. The dead time of this filter is ~1.8 ms when the corner frequency (f_c) was set to 100 Hz, and the data were sampled at an interval near the filter dead time. Under these conditions using a 50% threshold criterion for event detection, events with durations shorter than the filter dead time were never detected. Events longer than ~2.3 ms could be reliably detected, but events with durations between the filter dead time and 2.3 ms could be detected only part of the time and their durations will be underestimated. We have not corrected for this sampling error, as it apparently had little effect on our analysis because omission of events up to 18 ms did not distort the probability distributions.

In some cases, a depolarizing solution containing 150 mM KCl and 10 Hepes was used to zero the cell resting potential and allow accurate measurement of the single-channel slope conductance (γ) and reversal potential (V_{Rev}) under conditions where the *trans*-patch potential was accurately known. The patch pipette contained 4.5 mM potassium which determined the observed reversal potential. We then used the measured conductance value as a standard to derive the membrane potential (V_M) from the measured single-channel current (i) when the cells were not depolarized.

$$V_M = [i/\gamma] + V_{Rev}. \quad (1)$$

In five experiments where the bath and pipette potassium concentrations were 4.5 mM, the average resting potential obtained in this fashion was −70.3 ± 4.5 mV (23°C). In six additional cells in this external solution where the patch was broken, the measured resting potential was −73.8 ± 6.4 mV. Therefore, membrane potentials are reported as an absolute *trans*-patch potential.

After obtaining a GΩ seal, control data were collected for up to 30 min, then quinidine (10–50 μM) was superfused at 1 ml per min through a 0.5-ml bath volume and the channel activity was monitored by computer. Whenever possible, the drug was washed out of the bath to obtain a postdrug control. This usually required 15–20 min of reexposure to control solutions, and the drug effects were only partially reversible.

Because of the criteria that we established for making measurements, we have reported results from 9 out of the 130 membrane patches obtained in this series of experiments. The most common reason for rejecting a patch for further analysis was that the seal resistance was <100 GΩ, or that the GΩ seal was lost before completion of an experiment (70%). However, a significant number of patches could not be used because of the presence of too many (>6) channels (4% of patches), because inward rectifier channels were not detected (15% of patches), or because of disappearance of the K channel activity during the control period (4%). In addition, some patches were not included in the analysis due to the presence of channels other than the inward rectifier (7%).

Data analysis

To assess the steady-state probability of channel opening at different membrane potentials, patches were repeatedly clamped to test potentials 40, 70, or 100 mV negative to the resting potential for periods from 4–8 s. Because the probability of channel opening is nonstationary during the first 200–300 m, the first 200 sample points (400–800 m) after the voltage step were omitted from the steady-state analysis. Thus, the channel opening probabilities reported reflect only the steady-state conditions and not the initial peak opening probabilities. During the control and drug exposure periods, between 16 and 64 test pulses were applied at each membrane potential tested. Data from

multiple experiments are reported as means plus or minus one standard error of the mean (mean ± SEM). Differences between control and drug measurements were analyzed using a paired Student's *t*-test.

Data were analyzed with custom software written in BASIC (Microsoft, QuickBASIC) using an event detection scheme based on a half-amplitude criterion as described by Colquhoun and Sigworth (1983). Single channel recordings were first analyzed by generation of all points amplitude histograms which allowed identification of current levels associated with closed, substate, and full amplitude openings. These mean current levels were then used in the following event detection scheme. An event was registered if two consecutive digitized samples fell within a predetermined window of current levels. As described by Sakmann and Trube (1984*a*), at least four substate conductance levels exist for inward rectifier openings; hence, to evaluate the probability of at least one channel opening to any level (main or substate openings, the threshold for event detection was set to one half the amplitude of the lowest amplitude substate current level. To determine the probability of substate openings alone, the same lower threshold was used and an upper limit for event detection was imposed to eliminate current levels above the highest substate level (thereby eliminating main-state openings). For patches with more than one channel, we also calculated the probability of single main state openings by setting the detection window above and below the main current level to eliminate both substate openings and multiple simultaneous channel openings; such single mainstate opening probabilities could then be compared with the probability of substate openings below the mainstate. Window limits were optimized by visual inspection.

Estimates for the open-channel probability, including the individual probabilities of main and substate openings, were calculated as the fraction of time a channel spent at each current level. Although several substate levels exist, it was not possible to reliably quantify differential drug effects on the individual substate levels due to their low probability of occurrence. This problem was exacerbated in some cases by the limited duration over which data could be collected. Hence, in all cases the "substate probability of opening" refers to the lumped probability of all substate openings below the main amplitude level. In patches with more than one channel, the event detection scheme could provide the overall probability that at least one channel was open by keeping track of the fraction of time the current was above the threshold for detection of an open channel. To derive the probability of opening of a single channel, we assumed that the opening of different channels were not mutually exclusive events and therefore obeyed the summation law for the probability of the union of N events (Ross, 1972) where N is the number of channels and an event is a channel opening as follows.

$$\begin{aligned} P(&\text{at least 1 channel open when } N \\ &\text{channels are present}) \\ &= \Sigma\,\{\text{probability of } N \text{ channels} \\ &\quad \text{opening taken 1 at a time}\} \\ &\quad -\Sigma\,\{\text{probability of } N \text{ channels} \\ &\quad \text{opening taken 2 at a time}\} \\ &\quad +\Sigma\,\{\text{probability of } N \text{ channels} \\ &\quad \text{opening taken 3 at a time}\} \\ &\quad -\ldots+\Sigma\,\{\text{probability of } N \text{ channels} \\ &\quad \text{opening taken } N \text{ at a time}\}. \end{aligned} \quad (2)$$

We assumed that channels were functionally independent and had equal opening probabilities (Colquhoun and Sigworth, 1983). Hence, the probability (P) of at least one channel opening when four are present is as follows (p is the probability of one channel opening and the sum of n events taken r at the time is $n!/[(n-r)!r!]$):

$$P_{(\text{at least one channel open})} = 4p - 6p^2 + 4p^3 - p^4. \quad (3)$$

Because P was known, it was possible to solve numerically for the roots of the polynomial equations to provide the value of p (between 0 and 1). In each case only one such solution was found. The value of N for each experiment was estimated by counting the maximum number of channels open simultaneously during the drug-free period (usually at the beginning of each hyperpolarizing step). Others have found that this simple counting method works as well as more complicated statistical determinations of channel number (Aldrich et al., 1983; Sakmann and Trube, 1984*b*).

Examination of the amplitude histograms revealed that in many cases the peaks were poorly separated from each other causing difficulty in calculating the area under the peaks for analysis of probabilities. Therefore, we also analyzed the data using a second method which utilizes a mean-variance analysis recently described by Patlak (1988). The technique involves calculating a moving average current and variance in a window of 5–20 data samples. The window is advanced one sample point and a new mean and variance are calculated. The sample variance is relatively low when the channel is open or when it is closed, but during transitions from closed to open (or vice versa) the variance is high. A graph of this variance as a function of the mean current (Fig 4 *a*) results in a family of parabolas that arc between regions of low variance; these low-variance points typically indicate legitimate open and closed channel-current levels, while high-variance points represent either transitions between levels or very brief events. Whereas this technique clarifies the separation between current levels, the relative probabilities of the various conductance states may be distorted because samples are excluded from the analysis and thus the areas under the amplitude histograms may be decreased. In contrast, although there are uncertainties associated with the choice of levels in the "levels detection" method, the relative probabilities of occupying particular levels are less distorted by this technique (although picking out the levels can be more difficult).

In patches with more than one channel, mean open times were determined by using a first-open, first-closed scheme. This method of making closing assignments, although in principle a biased estimator, was used because it gave results similar to methods using random assignment of a closing to an opening and was simpler. Because the probability of channel opening was relative low (< 0.3) when using 4.5 mM external potassium, and because the substate openings were relatively rare in many cases, the open times were analyzed for all the open states of the channel as a group to provide sufficient numbers of events. Hence, occasional openings to substate levels were considered openings and were not distinguished from main-state openings for purposes of open time analysis (similar to Sakmann and Trube, 1984*b*). Events which opened to a substate level and then to the main-state level were analyzed as one event; a new event occurred either when full closure occurred or when the current level exceeded the threshold for a second opening. Because the open-time distributions were well approximated by a single exponential (see Results), then either the substate openings in these cases were not frequent enough to cause a detectable second exponential (also noted by Sakmann and Trube, 1984*b*), or the mean open times of the substate events were similar to that of the main state. Because the openings were described by a single exponential, the maximum likelihood estimate (arithmetic mean) of the mean open time was also calculated. This method of estimating the mean open times was preferable to exponential fitting because it avoided some of the pitfalls of binning the observations (Colquhoun and Sigworth, 1983).

Data were fitted using methods described in detail elsewhere (Balser et al., 1990). A modified SIMPLEX algorithm (Nelder and

Mead, 1965) for minimizing the residual sum of squares was used. All analysis of the effects of quinidine were obtained from paired observations.

RESULTS

Because most of these experiments were conducted in cells that were not exposed to elevated potassium, we were concerned about the uncertainty of the resting membrane potential in nondepolarized cells. Therefore, as described above, voltage ramps were used to determine the single-channel conductance and then Eq. 1 was used to estimate the resting membrane potential. Fig. 1 (*top*) shows current recorded during voltage ramps from 150–0 mV. The cell was depolarized in 150 mM KCl, therefore, the clamp potential should equal the *trans*-patch membrane potential and thus allow an accurate determination of the single-channel conductance. The currents elicited from two of the voltage ramps are shown. The channel was primarily open below −80 mV in one case, with only brief closures, but was fully closed

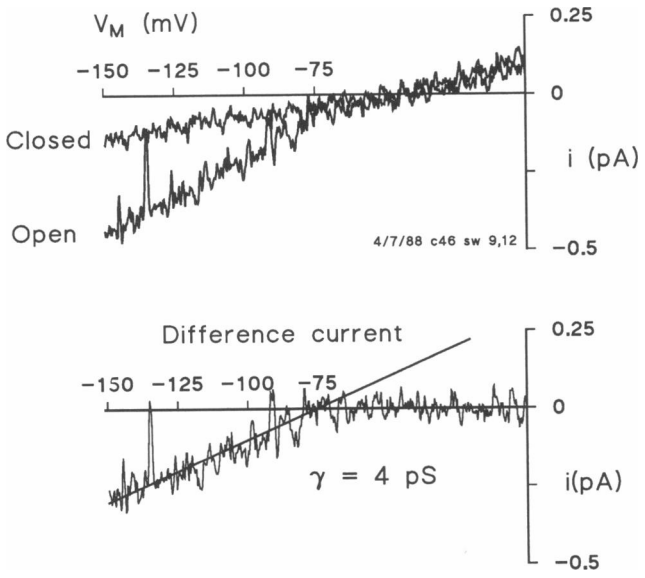


FIGURE 1 Single-channel current during voltage ramps from −150–0 mV. The cell was depolarized in 150 mM KCl which allowed accurate measurement of the single-channel slope conductance (γ) and reversal potential (V_{Rev}). The patch pipette contained 4.5 mM KCl. (*Top*) Two different records, one recorded when the channel was closed, and another recorded when the channel was open at −150 mV and remained open throughout the ramp (except for brief closures). (*Bottom*) The leak corrected single-channel K current. The averaged background current was subtracted from the record with the channel open. The slope conductance (γ) was obtained by fitting a line to the current elicited at membrane potentials between 130 and −90 mV. The zero current potential (*x*-intercept or V_{Rev}) was −77 mV.

throughout the voltage ramp in the other case. Slope conductances were estimated from the linear least squares regression on the difference current (Fig. 1, *bottom*) measured between −90 and −130 mV. Although the open-channel current-voltage relationship is not perfectly linear over an extended range of potentials, deviations from linearity were extremely small over the range we have used. The reversal potential was estimated from the *x*-intercept of the fitted line. The single-channel conductance was 3.9 ± 0.3 pS (n = 3) and the average reversal potential was −79.5 ± 2 mV. As reported by others (Sakmann and Trube, 1984*a*; Vandenberg, 1987; Matsuda et al., 1987) we observed strong inward rectification of this channel in the cell-attached mode (the difference current was negligible at membrane potentials more positive than −80 mV).

Fig. 2*a* shows representative channel activity recorded from an on-cell patch on a nondepolarized cell; both the pipette and bath solutions contained 4.5 mM KCl. The patch was voltage clamped to −174 mV from the resting potential of −74 mV. There were single openings to the main conductance level (1 open), double openings (2 open), and some additional less frequent openings to lower conductance levels (*a* substate, *b* substate, and *c* substate). These did not result from a separate class of K channels, but reflect openings of the inward rectifier K channel into a lower conductance substate conformation and have been observed by others (Sakmann and Trube, 1984a; Josephson and Brown, 1986). These lower conductance openings were 1/4, 1/2, and 3/4 of the main current level. We have observed in patches with only one channel that these current levels never exceeded the main (3.9 pS) conductance level as they would for separate channels if the lower amplitude openings were superimposed upon the main conductance. For a single channel with substates, occupancy of the various open states is mutually exclusive and overlap does not occur. For example, in an experiment where substate openings were especially prominent and only one apparent channel was observed, the probability of opening was 0.017 for the 3.9 pS events whereas the probability of all lower amplitude events was 0.007. If these events represented separate channels, the likelihood of observing simultaneous openings during the period recorded (more than 40 min) was >0.999. Because no simultaneous openings were ever observed, it was concluded that the events did not result from separate channels. This is consistent with the findings of Sakmann and Trube (1984*a*).

As shown in Fig. 2 *b*, 50 μM quinidine caused a decrease in channel activity that was partially reversible when quinidine was removed. The effect of quinidine was to substantially reduce the probability of channel opening, with no effect on the magnitude of the unitary current. In seven experiments (see Table 1), the mean

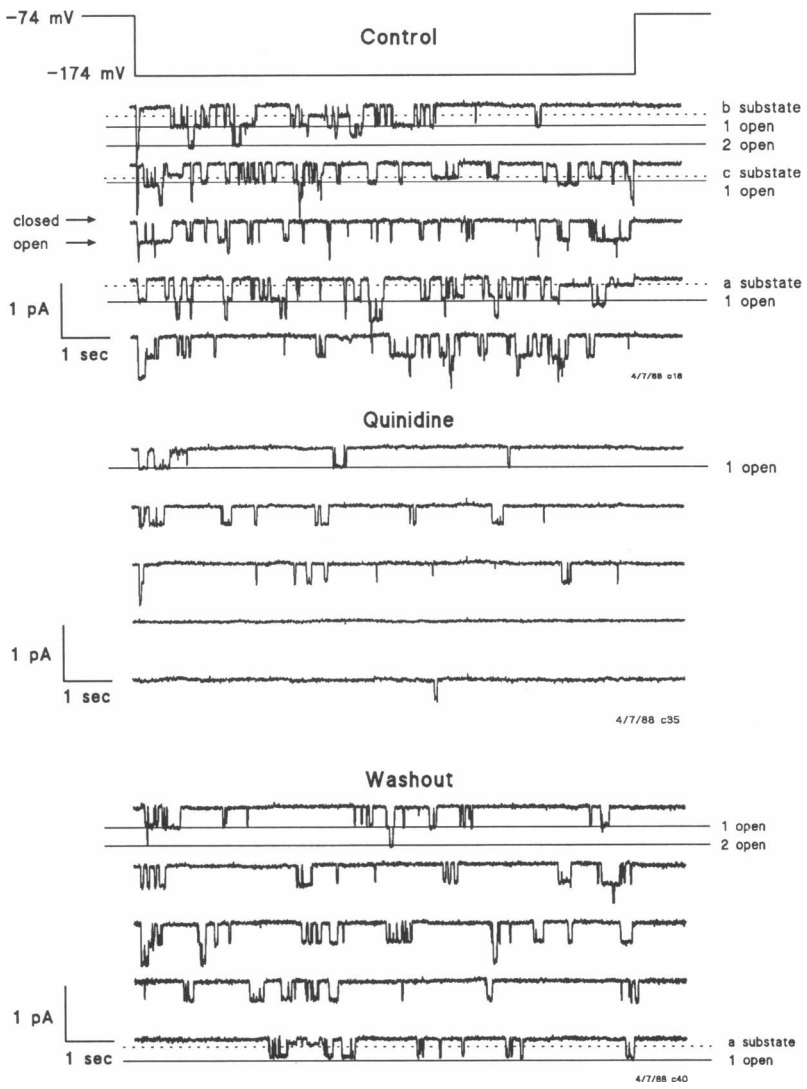


FIGURE 2 Reversible inhibition of single-channel activity by quinidine. (*a*) Five representative records obtained during 8-s steps to −174 mV in control. Pipette [K] = 4.5 mM, bath [K] = 4.5 mM. There are four channels in the patch. Sample rate 250 Hz, filter 50 Hz. The main unitary current level associated with single openings is indicated by the line labeled: 1 open. Also shown are the current levels associated with double openings (2 open), and three levels of subconductance openings (*a* substate, *b* substate and *c* substate). (*b*) The number of openings was reduced after 15 min of superfusion with 50 μM quinidine. The subconductance openings were eliminated. (*c*) The frequency of openings increased again after 20 min of drug wash-out and subconductance openings were seen again.

ratio of the single-channel current during drug exposure to that before drug exposure was 1.00 ± .024; in contrast, the probability of opening was reduced in six out of seven experiments. Note that for the probabilities of channel opening given in Table 1, the probabilities of opening to the main conductance state and lower conductance substates were combined. The probability of single channel opening for multichannel patches was calculated using Eqs. 2 as described above. The effect of quinidine shown in Fig. 2 *b* occurred after an exposure of ~10 min; note that single and double openings were present, but there were no substate openings. The drug effect was partially reversible 20 min after beginning to wash quinidine from the bath (Fig. 2 *c*): both the substate openings (*arrow*), as well as double openings were again observed.

If quinidine acts as an open-channel blocker (Armstrong, 1966), then it should shorten the time the channel spends in the open state by terminating the openings when block occurs. We tested whether quinidine was an open-channel blocker by directly observing the lifetimes of the open channel. The distribution of

TABLE 1 Effects of quinidine on single K channels

Cell	N^*	$[K]_{bath}$	$[K]_{pipette}$	$V^{\ddagger}_{rest}$	V_m	$i^{\S}_{cont}$	$i^{\S}_{quin}$	$i_{quin}/i^{\|}_{cont}$	P_{cont}	P_{quin}	[Quin]
		mM	*mM*	*mV*	*mV*	*pA*	*pA*				*μM*
4/13	6	150	4.5	0	−140	−0.22	−0.20	0.91	0.079	0.022	10
4/07	4	4.5	4.5	−74	−144	−0.25	−0.24	0.96	0.077	0.010	50
5/6a	2	4.5	4.5	−56	−126	−0.18	−0.18	1.00	0.042	0.029	50
5/6b	1	4.5	4.5	−61	−131	−0.20	−0.20	1.00	0.025	0.017	50
8/11	1	4.5	4.5	−77	−147	−0.26	−0.29	1.12	0.152	0.097	50
8/12	3	4.5	4.5	−82	−152	−0.28	−0.28	1.00	0.139	0.167	50
8/09	1	4.5	150	−70	−70	−1.74	−1.80	1.03	0.698	0.462	50

*Number of channels in the patch; ‡mean V_{rest} (from cells 2–6) = −70.3 ± 4.9 mV; $N = 5$; §main conductance level openings only; ‖mean i_{quin}/i_{cont} = 1.0 ± 0.02; $N = 7$.

open times was well described by a single exponential before and during quinidine exposure. Fig. 3 summarizes the open times obtained in several paired experiments, quinidine did not significantly alter the open lifetimes at the membrane potentials tested (−96 to −177 mV). The ratios of the paired open times are plotted in the upper graph and the mean ratio was not significantly different from unity. The grouped control open times, averaged over a narrow voltage range, were (μ ± SE): 249 ± 60 ms (−96 to −117 mV; $n = 4$), 118 ± 23 ms (−126 to −147; $n = 5$), and 82 ± 1 ms (−174 to −177; $n = 2$). The corresponding grouped mean open times from paired experiments in quinidine were 198 ± 48 ms, 119 ± 53 ms, and 54 ± 2 ms. As others have observed (Sakmann and Trube, 1984*b*) in elevated pipette potassium concentrations, the mean open time decreased as the membrane potential became more negative.

We estimated the effects of quinidine on the probabilities of channel opening using two techniques (see Methods). Fig. 4 shows an analysis of the conductance levels for cell 4 (Tables 1 and 2) at −131 mV; this patch contained only one channel. Fig. 4 *a* shows the variance (ordinate) as a function of the mean current (abscissa) in each of 4 different voltage-clamp steps. These histograms (Fig. 4 *b* and *c*) included only those points having a variance less than or equal to the background variance. The low-variance points indicate levels of current associated with closed, substate openings, and main state openings; the high-variance points are associated with sample points during transitions between conductance levels or very brief openings and closures. In this experiment only events > 18 ms had low enough (background) variance to be included in the analysis. Fig. 4 *b* shows the amplitude histogram when all sample points from 64 sweeps are included (*dotted line*) and when sample points having a variance exceeding the background variance are excluded. Note that excluding the high-variance points clarifies the divisions between the conductance levels. Fig. 4 *c* shows the amplitude histogram for the same cell after 15 min of quinidine (50 μM) exposure. Quinidine reduced the probability (area under the curve) of opening to the main conductance level by 20%, whereas the probability of substate openings was reduced by 98%.

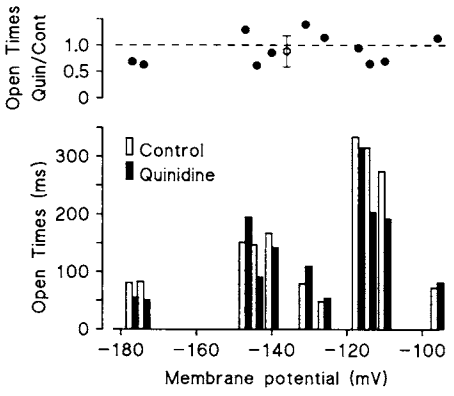


FIGURE 3 Effects of quinidine on open channel lifetimes. Summary of results from five experiments measuring open times at various membrane potentials. Open bars indicate control open times, closed bars indicate the presence of quinidine. The top panel shows the ratios of the mean open times in quinidine to the mean open times in control. A ratio of 1.0 indicates no effect. The mean and standard deviation of all ratios is shown as the open circle plotted in the middle of the voltage range.

Fig. 5 summarizes results from five patches (cells 1–5). In the lower panel, the probability of single-channel opening (both substates and the main open state) is shown as a function of membrane potential before (*open bars*) and during quinidine superfusion (*solid bars*). For each patch, the effects of quinidine were assessed at up to three different membrane potentials (as patch longevity would permit); each pair of bars (*open, solid*) in Fig. 5 thus represents a paired observation of the effect of quinidine at a particular membrane potential. Quinidine reduced P_{open} in all cases except at −96 mV, and in one additional membrane patch where the overall probability of channel opening was increased due to an increase in subconductance openings (data not shown in Fig 3,

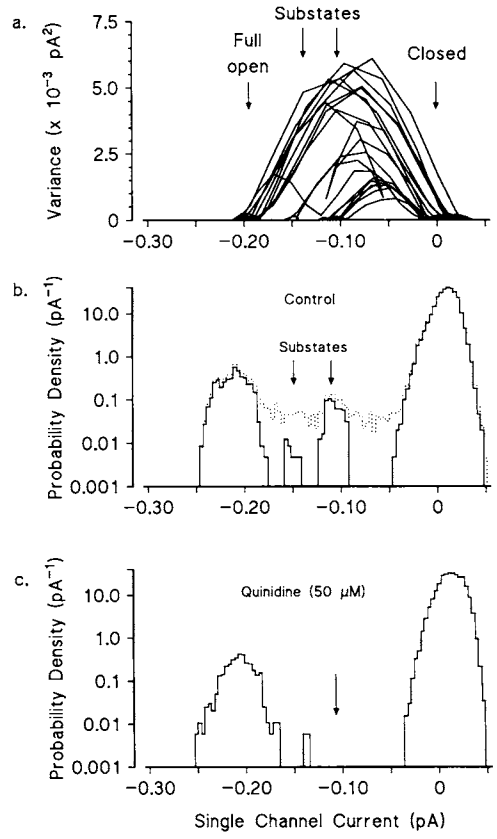


FIGURE 4 Effects of quinidine on single-channel conductance and substates. (*a*) Mean-variance plot of four control sweeps at −131 mV. The plot shows at least four levels associated with low variance, including closed channels, fully open channels, and two substate levels. Transitions between levels are shown as points with higher variance. A moving window of five sample points was utilized. (*b*) Amplitude histograms for 64 control sweeps from the same experiment at −131 mV. The dotted line indicates the histogram when all points are utilized; the solid line indicates the amplitude histogram when only sample points with a variance less than or equal to the background variance were included. The relative number of samples per bin (probability density) is plotted on a logarithmic ordinate as a function of current level of each sample. A peak in the histogram indicates a predominant current level. The peak centered at zero picoamperes indicates the closed channel current level. Other peaks indicate the amplitude (on the *x*-axis) of current through an open state of the channel. Notably the substate peaks are more easily distinguished when high-variance points are excluded. Bin width = 0.0035 pA. (*c*) Single-channel current amplitude histogram at −131 mV in the presence of 50 μM quinidine. Again, sample points with variance greater than background variance were excluded. In this case, quinidine reduced the probability of full openings by 20.1% and substate openings by 98.2% (Table 2).

cell 6 in Table 1). The top panel of Fig. 5 shows the ratio of P_{open} in quinidine relative to control. A ratio of unity indicates no effect. The results from both the levels analysis technique (*solid circles*) and from areas in amplitude histograms after mean-variance analysis (*open circles*) are shown. Quinidine reduced P_{open} and there was a voltage-dependent trend toward more block at more negative membrane potentials. The solid line represents a least squares linear regression to the data represented by the solid symbols. The fitted slope was significantly different from zero ($P < 0.05$). The dashed line indicates a similar regression line through the open circles with a slope also significantly different from zero ($P < 0.01$). Thus, both methods of obtaining P_{open} gave a similar result.

In 4/4 experiments where substate openings were prominent in control, the probability of substate openings was consistently reduced more than probability of main state openings (Table 2). An exception to this trend was found in one experiment (cell 6 in Tables 1 and 2). In this experiment, no substate openings were observed before drug exposure; however, lower amplitude openings were observed after drug exposure. Although the probability of mainstate opening was reduced, the overall probability of channel opening was in fact increased because of the appearance of these additional openings (hence, the apparent increase in the probability of channel opening for cell 6 in Table 1). Whereas we cannot readily account for this difference, it appears consistent with a recent brief report from other investigators (Sato et al., 1989). In our experiments, however, in all cases the single channel current amplitude histograms indicated that the main conductance peaks were not shifted by quinidine.

We frequently observed brief periods during which channel openings were absent even without quinidine (hereafter referred to as null episodes) which typically lasted less than 2 min. These null episodes suggested that the channel occasionally entered a state from which opening was unlikely. We tested whether the effect of quinidine was to increase the number of null episodes, and thereby promote occupancy of such a state. In paired observations from three patches containing only one channel, the fraction of episodes without openings was increased from 0.29 ± 0.2 in control to 0.43 ± 0.1 in quinidine ($p < 0.05$). In addition, quinidine (50 μM) also reduced the conditional probability of channel opening in episodes that contained openings by 14 ± 7% ($p < 0.05$). Hence, an increase in null episodes accounted for a proportion of quinidine's effect to reduce the probability of channel opening; however, the conditional probability of opening in episodes with openings also decreased.

DISCUSSION

We have investigated the inhibition of current through single inward rectifier K channels by quinidine in guinea pig ventricular myocytes. Channel permeation, as shown

TABLE 2 **Effects of quinidine on the fractional open time of the main- and sub-conductance levels**

Cell	V_M	Control		Quinidine		% decrease	
		F_{main}	F_{sub}	F_{main}	F_{sub}	F_{main}	F_{sub}
1. 4/13	−140	0.053	0.064	0.058	0.015	−10	76
2. 4/07	−144	0.100	0.027	0.019	0.002	81	91
3. 5/6a	−126	0.009	0.023	0.016	0.021	−86	8
4. 5/6b	−131	0.015	0.008	0.012	0.0001	20	98
6. 8/12	−152	0.237	0.000	0.166	0.114	30	++

F_{main} = fraction of time that one channel was open at the main conductance level; F_{sub} = fraction of time that one channel was open at nonzero current levels below the main conductance level. Patch 4 contained only one channel, therefore these values refer to the probability of channel opening to the main or subconductance levels, respectively. For the other multichannel patches, these values refer to the conditional probability that one channel is open to the main or subconductance levels given that there were N channels present in the patch. Cell 1, 10 μM quinidine, $[K]_{bath}$ = 150 mM; cells 2–4, 6, 50 μM quinidine, $[K]_{bath}$ = 4.5 mM; cells 5, 7, no substate openings in control or during quinidine; ++ substate openings were observed in quinidine but not in control.

by Armstrong (1971) for TEA block of delayed rectifier K channels in nerve, may modulate the characteristics of channel block (see also Cahalan and Almers, 1979). Therefore, we used on-cell patches and exposed the external side of the channel to 4.5 mM potassium, instead of the elevated potassium concentrations used in earlier studies (Sakmann and Trube, 1984*a* and *b*; Trube and Hescheler, 1984; Kurachi, 1985; Matsuda, 1988). Sakmann and Trube (1984*a*) observed a power law relationship between single K channel conductance (γ) and potassium concentration over the 11–145 mM concentration range they studied. By extrapolation of this relationship ($\gamma = 1.3[K^+]_o^{0.62}$ pS), the single-channel conductance should be ~3.3 pS in 4.5 mM external potassium. Using 10 mM K in the patch pipette, Mazzanti and DeFelice (1989) measured a conductance of ~14 pS (from their Fig. 10), and extrapolated their conductance data (10–120 mM K) to 2–3 pS for 1.3–2.5 mM K. Hence, our measurement of 3.9 ± 0.3 pS in 4 mM $[K]_o$ agrees fairly well with the predictions of other investigators. The main effect of quinidine observed on the channel activity was reduction of the probability of channel opening. These results are similar to those reported by Josephson (1988) for lidocaine effects on inward rectifier K channels; in that study, lidocaine reduced the probability of channel opening with no effect on the single-channel conductance.

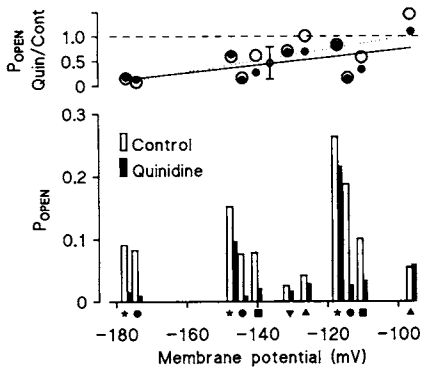


FIGURE 5 Effects of quinidine of K channel opening probability. (*Bottom*) The probability of single-channel opening (both substates and the main open state) is shown as a function of membrane potential from five experiments at one or more membrane potentials. Each pair of bars (*open, solid*) in the bottom panel represents a paired observation of P_{open} before and during quinidine at a particular membrane potential. Four of the experiments shown used 50 μM quinidine and one was in 10 μM quinidine. Because multiple voltages were examined in some experiments, the following symbols indicate the cell number in Table 1: squares, cell 1; circles, cell 2; triangles, cell 3; inverted triangles, cell 4; stars, cell 5. (*Top*) Ratios of P_{open} in quinidine to P_{open} in control. A ratio of 1.0 indicates no effect. Solid circles show the data from Fig 3 *a*; the mean and standard deviation of all ratios is shown as the open circle plotted in the middle of the voltage range. The solid line through the data represents a least squares linear regression with a slope that was significantly different from zero ($p < 0.05$). The larger open circles show the results when the probabilities were calculated from histograms derived from mean-variance analysis (see Methods). The dotted line shows the least squares linear regression of these data points; this slope was also significantly different from zero ($p < 0.01$).

Quinidine had no effect on the unitary current amplitude or on the duration of channel openings. Open-channel block as described for TEA^+ effects on delayed rectifier K channels by Armstrong (1966; squid axons) and Spruce et al. (1987; frog skeletal muscle), is usually expected to either reduce the mean open time of the channel or reduce the apparent single-channel conductance (fast-flicker block). Rather, quinidine appears to act by reducing the likelihood that channels will open. Because we have used on-cell patches to preserve the natural internal environment of the channel, our experiments cannot resolve whether this is a direct effect of quinidine on the channel protein. The effects of quinidine were not immediate, sometimes taking as long as 15 min after exposure to the bath solutions. Because the external surface of a channel faces the electrode solu-

tion, which is initially drug free, the drug must first diffuse into the cell to gain access to the channel. More than 90% of the quinidine molecules are charged at physiological pH, therefore entry into the cell is expected to be slow. After entering the cell, quinidine may bind to a portion of the channel exposed to the cytoplasm, or it could diffuse back through the patch of membrane into the electrode and then bind to the outer surface of the channel, although this seems less likely. Alternatively, once in the cell, quinidine could interfere with a cytoplasmic factor important for channel activation, or diffuse into the membrane and directly inhibit the channels by binding in the lipid phase. Any of these mechanisms could explain the relatively slow onset of the drug effect. We observed considerable variability in the magnitudes of drug effects (Tables 1 and 2; Fig. 3). It appears that this variability relates to quinidine's mechanism of action on the K channel in intact cells. If channel regulation or block are linked to membrane bound or cytoplasmic factors such as G-proteins or cAMP for example, the variability in channel gating and block could be explained by the variability in these uncontrolled systems. Quinidine is known to reduce cyclic AMP levels in guinea pig atrial cells (Mirro, 1981) which would be consistent with an indirect effect of quinidine if the channels were modulated by cyclic AMP, although there is no direct evidence for this at present. Additional experimentation will be required to discriminate these possibilities.

The effects of quinidine were only partially reversible (Fig. 2). Our observations are probably not inconsistent with the apparent irreversible quinidine-induced suppression of time-independent whole-cell inward current (Salata and Wasserstrom, 1988). Notably, inward rectifier K channel activity may cease abruptly and indefinitely in excised patches. Such a loss of channel activity is difficult to separate from a drug effect, but it occurs in the absence of a drug also. If a loss of channel activity occurred in the dialyzed whole-cell experiments during exposure to quinidine, such an effect might be erroneously attributed to the drug. In our experiments, suppression of channel openings by quinidine were clearly reversible upon removal of the drug. Further, although inward rectifier channels may cease functioning spontaneously, until this occurred the inward rectifier channel behavior appeared temporally stable. Three of the patches reported (cells 4, 5, and 7 in Table 1) contained only one channel and in all three cases, the likelihood of channel opening was reduced by quinidine. Hence, spontaneous loss of channel activity cannot explain the results from these single-channel patches.

Salata and Wasserstrom (1988) did not observe voltage or use-dependent suppression of whole-cell I_{KI}; however, their data were acquired only at potentials positive to −110 mV. Our data exhibited a voltage-dependent reduction of P_{open} by quinidine at membrane potentials between −100 and −180 mV (see Fig. 5). In most cases we observed a preferential reduction in the likelihood of substate openings in the presence of the drug; although the mechanistic implications of this observation are not precisely known, one possibility is that quinidine altered the free-energy barriers separating the open conformations of the channel protein. Thus, the channel was less likely to open to the main conductance level and even less likely to assume a substate conformation. In one experiment during quinidine exposure an increase in the occurrence of reduced amplitude openings was observed. This may represent some nonstationary behavior of the probability of substate openings that occurs over a time period longer than our ability to record the events. Alternatively, it could represent heterogeneity in the response to quinidine because similar effects have been observed by others (Sato et al., 1989) when using 140 mM potassium concentration in the patch pipette. We cannot distinguish these possibilities at the present time.

Trube and Hescheler (1984) reported that inward rectifier K channel activity often disappears after patch excision. We have also observed long periods (minutes up to an hour) without activity in cell-attached patches where a channel activity was previously observed; occasionally channel activity ceases and does not return. These observations suggest the possibility of an alternate gating mode, and may indicate that cytoplasmic or membrane-bound factors play an important role in the regulation of this channel. Similar long "nonactivatable" periods have also been noted for on-cell patches of single-cardiac Na channels (Horn et al., 1984; Kohlhardt et al., 1988); this Na channel behavior has also been interpreted as a spontaneously occurring "nonactivatable" gating mode (Kohlhardt et al., 1988). Because the effect of quinidine on single channel patches included a reduction in the conditional probability of channel opening in episodes (hyperpolarizing steps) that contained activity, it is clear that quinidine did not act exclusively by placing the channel in such a "nonactivatable" state. Further, although in some patches channel activity occasionally disappeared for long periods of time (minutes up to one hour), this was not correlated with the application of quinidine.

We have attempted to interpret our results within the context of the model proposed by Sakmann and Trube (1984*b*).

$$C_1 \underset{k_{12}}{\overset{k_{21}}{\rightleftharpoons}} C_2 \underset{k_{23}}{\overset{k_{32}}{\rightleftharpoons}} O_3.$$

This model could account for the brief closures

occurring between channel openings during hyperpolarizing steps as well as the longer closures associated with null episodes. This description is clearly an oversimplification because closed histograms suggest that there are more than two closed states (Sakmann and Trube, 1984*b*). Further, this model ignores the subconductance states of the channel (only one open state is utilized). However, it is a tractable model that can reasonably account for a majority of the observed kinetic features.

We first used the observed mean open times to fix the rate constant (k_{23}).

$$1/\tau_{open}(V) = k_{23}(V) = k_{23}(0) \cdot \exp^{(z\delta eV/kT)}, \quad (4)$$

where $k_{ij}(0)$ is the value of the rate constant in the absence of an electrical field, V is membrane potential, $z\delta$ is the fractional electrical field strength, e is the value of an elementary charge, k is the Boltzmann constant, and T is temperature. The value of kT/e is ~25 mV at 20°C. The fit to the control mean open times is shown in Fig. 6 (*upper panel*); the fitted value for $k_{23}(0)$ was 1.81 s^{-1} and $z\delta$ was −0.24. This left three additional rate constants to be estimated. The middle panel in Fig. 6 shows the fit of the C-C-O model to the steady-state control P_{open} data (shown in Fig. 3, *solid circles*) using the exponential voltage dependence of the rate constants

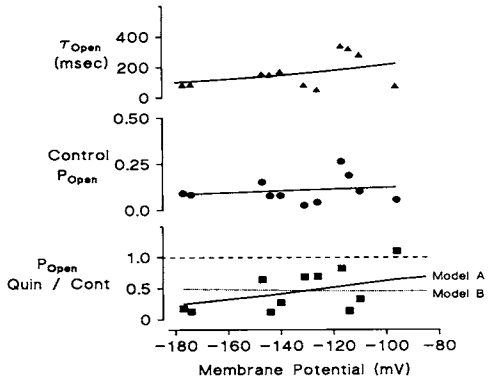


FIGURE 6 Kinetic model fitted to control and quinidine data. (*Top*) Least squares fit of voltage dependence of the mean open times (Eq. 4). The fitted values for $k_{23}(0)$ and $z\delta$ are given in the text. (*Middle*) Fit of the C-C-O model to the steady-state control P_{open} data using the voltage-dependence for the rate constants predicted by Eyring theory (see text). The fitted values for the rate constants are provided in the text. (*Bottom*) Fits of models A and B (see text) to the data from Fig. 4 (*top*) showing ratio of the probability of opening in quinidine to control. The fit to model A is shown by the solid line, model B by the dashed line. Note the apparent voltage dependence of the block is accounted for by model A even though the drug binding rate constants (k_{on} and k_{off}) were not voltage dependent. Model B failed to account for the apparent voltage dependence of block. The sum-of-squared error for models A and B with the same number of degrees of freedom were 0.0672 and 0.0715, respectively.

predicted from reaction rate theory (Eyring et al., 1980; Stevens, 1978). The fitted equations for the other three rate constants were as follows (k_{ij} in seconds^{-1}, V in millivolts):

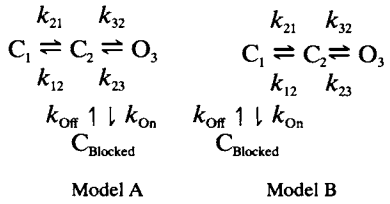
$$k_{12} = 46.99 \cdot \exp^{(+0.078e \cdot V/kT)} \quad (5)$$

$$k_{21} = 0.225 \cdot \exp^{(-0.486e \cdot V/kT)} \quad (6)$$

$$k_{32} = 74.44 \cdot \exp^{(+0.402e \cdot V/kT)} \quad (7)$$

The values of these rate constants at −110 mV differed from those previously determined by Sakmann and Trube (1984*b*) using this model; however, their rate constants (see their Fig. 9) give a calculated steady-state probability of channel opening that is higher than we observe experimentally. This could be due to differences in experimental conditions between their study and ours, especially with regard to external potassium concentrations (Cohen et al., 1989).

A major effect of quinidine was to increase the number of null episodes during hyperpolarization of the patch. The simplest modification to the model that could account for these effects of quinidine on the probability of channel opening was to add a drug-induced state to one of the two closed states, thereby creating an additional state with no direct connection to the open state. We excluded open channel interactions because the drug had no significant effect on the channel open times. If in fact quinidine acts by direct state-dependent binding to the channel, then the additional drug-induced state may be viewed as drug-associated closed ("blocked") state (Hondeghem and Katzung, 1977; Hille, 1977). We tested two possible models:



The rate constants k_{12}, k_{21}, k_{23}, and k_{32} were determined from the control data (*middle panel*, Fig. 6); therefore, only two rate constants remained to be estimated for each model (k_{Off} and k_{On}). We made the simplest assumption that these rate constants were not voltage dependent. Both models accounted equally well for the increase in the number of null episodes, however, only model A gave the appropriate voltage dependence for P_{open}. For model A, k_{Off} = 0.602 s^{-1} and k_{On} = 3.40 s^{-1} (k_a = 60,800 M^{-1} s^{-1}). For model B, k_{Off} = 0.918 s^{-1} and k_{On} = 1.275 s^{-1} (k_a = 25,500 M^{-1} s^{-1}). The bottom panel of Fig. 6 shows the ratios of the probability of opening in quinidine to control with the least-squares fits to models

A and B. Model B failed to account for the apparent voltage dependence of block and had a higher sum-of-squares error than model A (see figure legend). Because neither model is a subset the other (they are nonnested), simple expressions for statistical discrimination between the two models do not exist. It is possible to discriminate the models based on the log error ratio (LER; Akaike, 1974; Horn, 1987), however, significance levels cannot be calculated with this approach. The LER criterion indicated that model A was superior to model B. Statistical proof for the superiority of model A lies in more sophisticated methods for discrimination of nonnested models using Monte Carlo methods described by Horn (1987); such an analysis warrants further study but is beyond the scope of this paper. Therefore, the superiority of model A is not proven; however, based on both simple observation and on the LER criterion, model A seems more consistent with our data than model B. Fig. 7 compares single-channel data from a patch before and during exposure to quinidine (Fig. 7 *A*) with reconstructed single-channel and ensemble-average behavior (Fig. 7 *B*) of the control three-state model (7 *B, left*) and model A (7 B, *right*). If we assume a modulated receptor model of direct state-selective drug binding to state 2, then a K_D of ~10 μM is estimated for model A. However, this value is based on the bath concentration (50 μM) of quinidine and we do not know the concentration at the actual site of action. Furthermore, we have not assessed whether a clear concentration-dependence exists for these quinidine effects.

In summary, we have demonstrated that quinidine reduces current through inward rectifier K channels by decreasing the likelihood that a given single channel will open. The single-channel mechanism of block does not involve a reduction of the open-channel duration, or a decrease in the single-channel conductance. The results suggest that quinidine decreased the ability of the channel protein to enter either the main- or the subconducting conformations. This further suggests that quinidine may stabilize the channel in a closed (or "blocked") state and thus reduce the chances of channel opening. These observations were accounted for quantitatively by using the kinetic model proposed by Sakmann and Trube (1984*b*) and modified for quinidine binding to the middle closed state.

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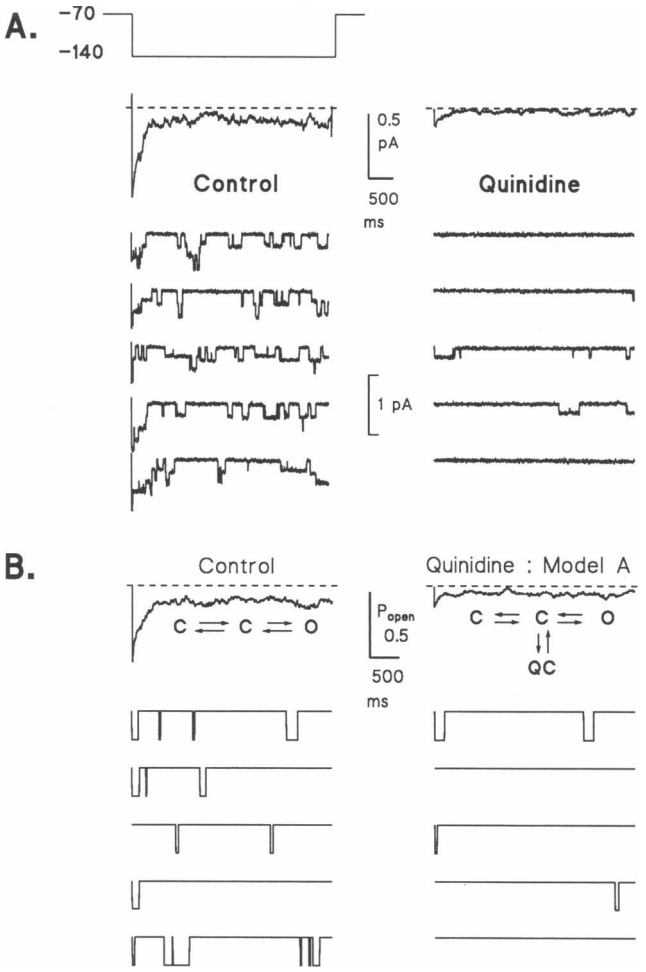

FIGURE 7 Simulation of ensemble and single channel gating behavior. (*A*) Left: Control channel openings recorded during voltage clamp steps to −140 mV. *Right:* similar steps recorded in quinidine. Ensemble averages are shown above each set of records. (*B*) Simulated single-channel openings during steps to −140 mV (*below*) and the corresponding ensemble averatges of 200 such steps (*above*). The simulations use the rate constants derived from fitting the C-C-O model to the data (*left*) and the quinidine model A (*right*). Because the simulations required steps from potentials near E_K where we had no data regarding P_{open}, for the C-C-O model P_{open} was set to be 0.62 at $t = 0$ (as determined in Sakmann and Trube, 1984*b*). The peak ensemble current in quinidine was 20% of the peak control ensemble current; thus, the initial P_{open} for the quinidine simulation was 0.124 (20% of 0.62)

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REFERENCES

Akaike, H. 1974. A new look at the statistical model identification. *IEEE (Inst. Electr. Electron. Eng.) Trans. Automatic Control.* AC19: 716–723.

Aldrich, R. W., D. P. Corey, and C. F. Stevens. 1983. A reinterpretation of mammalian sodium channel gating based on single channel recording. *Nature (Lond.)*. 306:436–441.

Armstrong, C. M. 1966. Time course of TEA^+-induced anomalous rectification in squid giant axons. *J. Gen. Physiol.* 50:491–503.

Armstrong, C. M. 1971. Interaction of the tetraethylammonium ion derivatives with the potassium channels of giant axons. *J. Gen. Physiol.* 58:413–437.

Balser, J. R., D. M. Roden, and P. B. Bennett. 1990. Global parameter optimization for cardiac potassium channel gating models. *Biophys. J.* 57:433–444.

Bennett, P. B. and T. B. Begenisich. 1987. Catecholamines modulate the delayed rectifying potassium current (I_K) in guinea pig ventricular myocytes. *Pfluegers Arch. Eur. J. Physiol.* 410:217–219.

Cahalan, M. D., and W. Almers. 1979. Interactions between quaternary lidocaine, the sodium channel gates and tetrodotoxin. *Biophys. J.* 27:39–56.

Cohen, I.S., D. DiFrancesco, N. K. Mulrine, and P. Pennefather. 1989. Internal and external K^+ help gate the inward rectifier. *Biophys. J.* 55:197–202.

Colatsky, T. J. 1982. Mechanisms of action of lidocaine and quinidine on action potential duration in rabbit cardiac Purkinje fibers. *Circ. Res.* 50:17–27.

Colquhoun, D., and F. J. Sigworth. 1983. Fitting and statistical analysis of single-channel records. *In* Single-Channel Recording. B. Sakmann, and E. Neher, editors. Plenum Publishing Corp., New York. 191–263.

Eyring, H., S. H. Lin, and S. M. Lin. 1980. Basic Chemical Kinetics. John Wiley & Sons Ltd. New York

Farmer, B. B., M. Mancina, E. Williams, and A. Watanabe. 1983. Isolation of calcium tolerant myocytes from adult rat heart: review of the literature and description of a method. *Life Sci.* 33:1–18.

Furukawa, T., Y. Tsujimura, K. Kitamura, H. Tanaka, and Y. Habuchi. 1989. Time- and voltage-dependent block of the delayed K^+ current by quinidine in rabbit sinoatrial and atrioventricular nodes. *J. Pharmacol. Exp. Ther.* 251:756–763.

Hamill, O. P., A. Marty, E. Neher, S. Sakmann, and F. J. Sigworth. 1981. Improved patch clamp techniques for high-resolution current recording in cells and cell-free membrane patches. *Pfluegers Arch. Eur. J. Physiol.* 391:85–100.

Hess, P., and R. W. Tsien. 1984. Mechanism of ion permeation through calcium channels. *Nature (Lond.)*. 309(31):453–456.

Hille, B. 1977. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen. Physiol.* 69:497–515.

Hiraoka, M., K. Sawada, and S. Kawano. (1986). Effects of quinidine on plateau currents of guinea-pig ventricular myocytes. *J. Mol. Cell Cardiol.* 18:1097–1106.

Hondeghem, L. M., and B. G. Katzung 1977. Time and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. *Biochim. Biophys. Acta.* 472:373–398.

Horn, R. 1987. Statistical methods for model discrimination. Application to gating kinetics and permeation of the acetylcholine receptor channel. *Biophys. J.* 51:255–263.

Horn, R., and C. A. Vandenberg. 1984. Statistical properties of single sodium channels. *J. Gen. Physiol.* 84:505–534.

Imaizumi, Y., and W. R. Giles. 1987. Quinidine-induced inhibition of transient outward current in cardiac muscle. *Am. J. Physiol.* 253: H704–H708.

Josephson, I. R. 1988. Lidocaine blocks Na, Ca and K currents of chick ventricular myocytes. *J. Mol. Cell. Cardiol.* 20:593–604.

Josephson, I. R., and A. M. Brown. 1986. Inwardly rectifying single-channel and whole cell K^+ Currents in rat ventricular myocytes. *J. Membr. Biol.* 94:19–35.

Kohlhardt, M., and H. Fichtner. 1988. Block of single cardiac Na^+ channels by antiarrhythmic drugs: the effect of amiodarone, propafenone, and diprafenone. *J. Membr. Biol.* 102:105–119.

Kurachi, Y. 1985. Voltage-dependent activation of the inward-rectifier potassium channel in the ventricular cell membrane of guinea-pig-heart. *J. Physiol. (Lond.)*. 366:365–385.

Matsuda, H. 1988. Open-state substructure of inwardly rectifying potassium channels revealed by magnesium block in guinea-pig heart cells. *J. Physiol. (Lond.)*. 237–258.

Matsuda, H., A. Saigusa, and H. Irisawa. 1987. Ohmic conductance through the inwardly rectifying K channel and blocking by internal Mg^{2+}. *Nature (Lond.)* 325:156–159.

Mazzanti, M., and L. J. DeFelice. 1988. K channel kinetics during the spontaneous heart beat in embryonic chick ventricle cells. *Biophys. J.* 54:1139–1148.

Mirro, M. J. 1981. Effects of quinidine, procainamide, and disopyramide on automaticity and cyclic AMP content of guinea-pig atria. *J. Mol. Cell. Cardiol.* 13:641–653.

Nelder, J. A., and R. Mead. 1965. A simplex method for function minimization. *Comput. J.* 7:308–313.

Patlak, J. B. 1988. Sodium channel subconductance levels measured with a new variance-mean analysis. *J. Gen. Physiol.* 92:413–430.

Roden, D. M., P. B. Bennett, D. J. Snyders, J. R. Balser, and L. M. Hondeghem. 1988. Quinidine delays I_K activation in guinea pig myocytes. *Circ. Res.* 62:1055–1058.

Ross, S. M. 1972. Introduction to Probability Models. Academic Press, New York.

Sakmann, B., and G. Trube. (1984*a*). Conductance properties of single inwardly rectifying potassium channels in ventricular cells from guinea-pig heart. *J. Physiol. (Lond.)*. 347:641–657.

Sakmann, B., and G. Trube (1984*b*). Voltage-dependent inactivation of inward-rectifying single-channel currents in the guinea-pig heart cell membrane. *J. Physiol. (Lond.)*. 347:659–683.

Salata, J. J., and J. A. Wasserstrom. 1988. Effects of quinidine on action potentials and ionic current in isolated canine ventricular myocytes. *Circ. Res.* 62:324–337.

Sato, R., I. Hisatome, J. A. Wasserstrom, and D. H. Singer. 1989. Mechanism of quinidine block of inward-rectifier K^+ channel (I_{K1}). *Biophys. J.* 55:44a. (Abstr.)

Spruce, A. E., N. B. Standen, and P. R. Stanfield. 1987. The action of external tetraethylammonium ions on united delayed rectifier potassium channels of frog skeletal muscle. *J. Physiol. (Lond.)*. 393:467–478.

Stevens, C. F. 1978. Interactions between intrinsic membrane protein and electric field. An approach to studying nerve excitability. *Biophys. J.* 22:295–306.

Trube, G., and J. Hescheler. 1984. Inward-rectifying channels in isolated patches of heart cell membrane: ATP-dependence and comparison with cell-attached patches. *Pfluegers Arch. Eur. J. Physiol.* 401:178–184.

Vandenberg, C. A. 1987. Inward rectification of a potassium channel in cardiac ventricular cells depends on internal magnesium ions. *Proc. Nat. Acad. Sci. USA.* 84:2560–2564.