

Action of Quinidine on Ionic Currents of Molluscan Pacemaker Neurons

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ABSTRACT The effects of quinidine on the fast, the delayed, and the Ca^{2+} -activated K^+ outward currents, as well as on Na^+ and Ca^{2+} inward currents, were studied at the soma membrane from neurons of the marine mollusk *Aplysia californica*. External quinidine blocks these current components but to different degrees. Its main effect is on the voltage-dependent, delayed K^+ current, and it resembles the block produced by quaternary ammonium ions (Armstrong, C. M., 1975, *Membranes, Lipid Bilayers and Biological Membranes: Dynamic Properties*, 3:325–358). The apparent dissociation constant is $28 \mu\text{M}$ at $V = +20 \text{ mV}$. The blocking action is voltage and time dependent and increases during maintained depolarization. The data are consistent with the block occurring $\sim 70\text{--}80\%$ through the membrane electric field. Internal injection of quinidine has an effect similar to that obtained after external application, but its time course of action is faster. External quinidine may therefore have to pass into or through the membrane to reach a blocking site. The Ca^{2+} -activated K^+ current is blocked by external quinidine at concentrations 20–50-fold higher compared with the delayed outward K^+ current. In addition, it prolongs the time course of decay of the Ca^{2+} -activated K^+ current. Na^+ and Ca^{2+} inward currents are also blocked by external quinidine, but again at higher concentrations. The effects of quinidine on membrane currents can be seen from its effect on action potentials and the conversion of repetitive “beating” discharge activity to “bursting” pacemaker activity.

INTRODUCTION

Quinidine and its stereoisomer quinine are prototypes of a category of compounds that are well known because of their antiarrhythmic action on excitable cells. From studies on the mechanism of action of these drugs, it was found that they suppress various membrane conductances in a variety of cells, such as squid axon (Yeh and Narahashi, 1976), *Myxicola* giant axon (Wong, 1981), molluscan neurons (Hermann and Gorman, 1981, 1983; Walden and Speckmann, 1981), cat motoneurons (Puil and Krnjevic, 1976), frog node of Ranvier (Revenko et

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al., 1982), neuroblastoma cells (Fishman and Spector, 1981), heart muscle (Ducouret, 1976; Nawrath, 1981), smooth muscle (Huddart and Saad, 1977), barnacle photoreceptors (Hanani and Shaw, 1977), and guinea pig hepatocytes (Burgess et al., 1981). We initially became interested in this drug because of reports of its specific blocking action on the Ca^{2+} -activated K^+ conductance in red blood cells (Armando-Hardy et al., 1975; Lew and Ferreira, 1978). In molluscan neurons, quinine appeared not to prevent the increase of K^+ permeability induced by intracellular Ca^{2+} injection (Meech, 1976). Subsequent studies under voltage clamp conditions revealed, however, that the drug blocks the Ca^{2+} -activated K^+ conductance, but its action appeared not to be specific to this current component (Thompson and Aldrich, 1980; Hermann and Gorman, 1981c, 1983; Walden and Speckmann, 1981). A comparative investigation of the blocking action of quinidine on various current components, to determine its primary site of action and its mechanism of action, was not carried out in detail. In particular, the rhythmogenic action of the drug is still poorly understood.

In this study we have compared the effect of quinidine on five specific ionic currents and the leakage current found in the molluscan neuron soma membrane. The primary action of the drug is on the delayed rectifier current, and it resembles the block produced by quaternary ammonium ions (Armstrong, 1975). At higher concentrations, quinidine suppresses the Ca^{2+} -activated K^+ current and, in addition, prolongs the subsidence of the remaining current. The effects of quinidine on the various membrane currents can be used to explain its effects on action potentials and the transformation of spontaneously "beating" into "bursting" discharge activity. Preliminary accounts of this work have been presented elsewhere (Hermann and Gorman, 1981c, 1983).

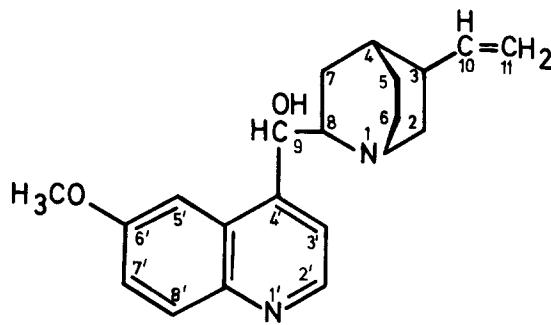
METHODS

Experiments were performed on identified cells in the abdominal ganglion of the marine mollusk *Aplysia californica*. In most cases the pacemaker cells R-15, L-11, or L2-L6 were used, but other cells gave similar results. The experimental procedures, recording, voltage clamp, and iontophoresis were identical to those described previously (Hermann and Gorman, 1981a). The abdominal ganglion was placed in a recording chamber containing artificial seawater (ASW) and desheathed to expose the cells. If possible, the cells were undercut to remove part of the axon and collaterals in an attempt to minimize the contribution of currents from unclamped regions of the cell. The ASW contained (in mM): 478 NaCl, 10 KCl, 10 CaCl_2 , 55 MgCl_2 , and 15 Tris-HCl at pH 7.6. In nominally Ca^{2+} -free ASW solution, Ca^{2+} was replaced by Mg^{2+} or Co^{2+} . In high K^+ solutions, Na^+ was replaced by K^+ . Tetrodotoxin (TTX) was added directly to the solution just before use. When external tetraethylammonium-bromide (TEA) (Fluka Chemical Co., Neu-Ulm, FRG) or 4-aminopyridine (4-AP) (Aldrich Chemical Co., Inc., Milwaukee, WI) was used, an equimolar amount of Na^+ was replaced.

Quinidine is the *d*-stereoisomer to quinine, and has the structural formula shown in Fig. 1. There are two ring forms in the structure of quinidine, the aromatic quinoline ring and the nonaromatic quinuclidine ring; both rings are linked by a secondary alcohol connection. In addition, there is a methoxy group in the quinoline ring and a vinyl group in the quinuclidine ring. The nitrogen atoms are both basic. Quinidine has a pK_1 of 8.77 and a pK_2 of 4.2 (pK_a) (Perrin, 1965). Quinidine-HCl (Sigma Chemical Co., St. Louis, MO)

solutions were freshly made from stock solutions before experiments. The exchange of the bath solution (chamber volume ~ 1 ml) was complete in 5–20 s (cf. Gorman and Hermann, 1979). The bath temperature was kept constant at 16°C. During solution exchange the temperature did not change more than $\pm 1^\circ\text{C}$.

The microelectrodes were filled with 3 M KCl solution and had resistances of 1–3 M Ω . Two intracellular electrodes were used to voltage clamp the cell and further electrodes were used for injections of Ca^{2+} , EGTA (Sigma Chemical Co.), or quinidine. The membrane potential was measured differentially with respect to an extracellular electrode that was independent of bath ground. Membrane currents were measured in the experimental bath with a virtual ground circuit. Intracellular injections were usually made ionophoretically into voltage clamped cells from electrodes containing 0.1 M Ca^{2+} , 0.5 M K-EGTA, or 5 mM quinidine. For injections, quinidine was dissolved in distilled water at a pH of 5. At this pH, virtually all of the drug is in its charged form. Nevertheless, injection quality was poor. Therefore quinidine was also pressure injected from pipettes containing the injection solution at pH = 7.3, i.e., close to the intracellular pH. There was no difference in the results obtained with either injection technique.



quinidine ($\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_2$)

FIGURE 1. Structure of quinidine.

Leakage currents were estimated from hyperpolarizing voltage pulses or from the initial jump in current that occurs after the capacitive current. In the current-voltage plots, leakage currents were subtracted. The series resistance is 1–3 k Ω in these cells (Hermann and Gorman, 1981a). A standard series resistance compensation of 1–1.5 k Ω was used. Membrane potential oscillations that damage the cell were avoided by a clamp shut-off control with a variable delay between 100 μs and 10 ms. Measurements were made on at least three cells and are given as means \pm standard error of the mean if not indicated otherwise. Records were taken on a three-channel rectilinear pen-recorder and on film.

The soma membrane of molluscan nerve cells exhibits a number of different inward and outward currents (Adams et al., 1980). In *Aplysia* neurons there are at least three types of outward currents carried by K^+ ions that are to various degrees voltage and time dependent. The fast outward K^+ current activates at membrane potentials more negative than about -40 mV, the delayed outward K^+ current activates at potentials more positive than about -20 mV, and the Ca^{2+} -activated K^+ current activates at negative and positive potentials but shows strong outward rectification. Furthermore, there are at least two voltage-dependent inward current components carried by Na and by Ca ions. A non-ion-

specific leakage current component is found at potentials negative to the membrane holding potential, which was usually -40 to -50 mV. The individual current components were separated as described in detail previously (Hermann and Gorman, 1981a) by pharmacological means, ion exchange, or by setting a certain membrane holding potential. The separation procedure is briefly indicated in the Results.

RESULTS

In the presence of external quinidine, inward as well as outward currents were suppressed but to different degrees. Table I summarizes values of apparent dissociation constants (K_Q), relating the effects of quinidine to the individual current components. Data are given for an external, 30-min application of the drug at the indicated membrane potentials. The reversibility of the drug effects is poor and therefore experiments were done by increasing drug concentrations without intermediate washouts. K_Q values were calculated assuming that the control values before the application of the drug remain constant. As it is possible to obtain a reasonable steady state of currents whose magnitude depends on the

TABLE I
Apparent Dissociation Constants (K_Q) for the Block of Various Membrane Current Components by Quinidine (Mean \pm SEM)

	I_{Na}	I_{Ca}	$I_{K,A}$	$I_{K,V}$	$I_{K,Ca}$
K_Q (μ M)	680 ± 290	260 ± 130	120 ± 35	28 ± 13	$1,400 \pm 160$
n	3	7	3	12	9
V (mV)	+20	+20	-20	+20	+20

I_{Na} is the fast Na^+ inward current, I_{Ca} is the Ca^{2+} inward current, $I_{K,A}$ is the voltage-activated, fast K^+ outward current, $I_{K,V}$ is the voltage-activated, delayed K^+ outward current, and $I_{K,Ca}$ is the Ca^{2+} -activated K^+ current elicited by ionophoretic Ca^{2+} injections. n indicates the number of experiments and V is the membrane potential.

drug concentration, a rundown of the cells caused by quinidine appears unlikely. The comparison of dissociation constants shows that quinidine predominantly affects the voltage-activated, delayed K^+ outward current. The fast K^+ outward current cannot be directly compared with other currents because of the negative potential range, where activation of this current in isolation is possible. It appears, however, that the dissociation constant falls within the values expected for the delayed K^+ outward current at a potential of -20 mV. The Ca^{2+} -activated K^+ outward current, the Ca^{2+} inward current, and the Na^+ inward current are blocked at 20–50-fold-higher concentrations of quinidine compared with the delayed K^+ current. The effects of quinidine on the delayed K^+ outward current have been studied in more detail.

Effects of External Quinidine on the Voltage-activated, Delayed K^+ Current

The voltage-activated, delayed K^+ outward current was separated from other current components by use of ASW solution containing 50μ M TTX to block the fast Na^+ inward current and 10 mM Co^{2+} , zero Ca^{2+} to block the Ca^{2+} inward

current and the Ca^{2+} -activated K^+ current. Furthermore, the membrane voltage was held at -40 to -45 mV to inactivate the fast K^+ outward current. The residual voltage-activated, delayed K^+ outward current recorded under these conditions rises rapidly to a maximum and thereafter slowly decays (Hermann and Gorman, 1981a, b). Intervals between steps were 1 min to provide for recovery from most of the inactivation.

Fig. 2 shows current traces before and after application of quinidine. At low

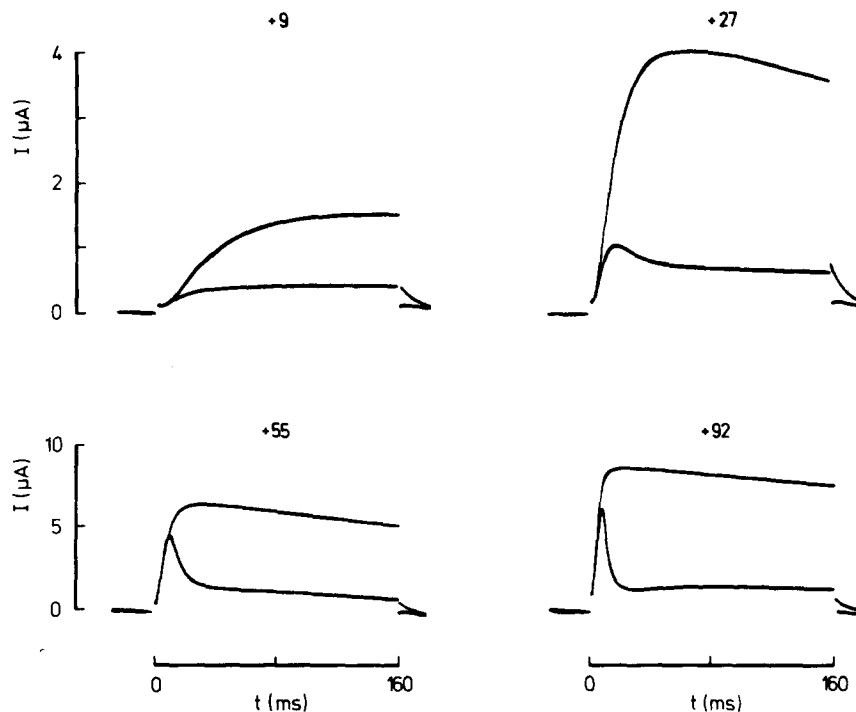


FIGURE 2. Effect of external quinidine on the voltage-activated, delayed K^+ outward current of the *Aplysia* neuron R-15. Membrane currents were recorded in ASW solution containing 10 mM Co^{2+} , 50 μM TTX, and zero Ca^{2+} . The cell was injected with EGTA (225 μC). The upper traces show outward currents under control conditions and the lower traces show the currents after application of 100 μM quinidine for 30 min. The membrane potential was clamped from a holding potential of -40 mV to the indicated voltages.

voltages (to $+9$ mV), there is only a reduction of the peak current, while the rise and decay phase are not much altered. At increasing positive membrane potentials, in addition to the reduction of the peak current, the decay phase of outward currents is more rapid. The rate of the decay of the current to a final level increases with more positive membrane potentials. At still higher voltages, the current does not decay to a plateau, but increases slightly again.

The rising phase of the outward K^+ currents appeared not much altered at

low concentrations of quinidine ($\leq 100 \mu\text{M}$). At higher concentrations of the drug, the activation time course of the outward currents was, however, somewhat decreased.

Tail currents at the end of a voltage step first increase and then decline in quinidine solution, whereas in control solution tail currents decline monotonically (Fig. 2). The decline in quinidine solution is also slower compared with that in control solution. Assuming a block of K^+ channels by quinidine, such a waveform of tail currents is expected if unblocking of channels is faster than closing of the channels (Shapiro, 1977).

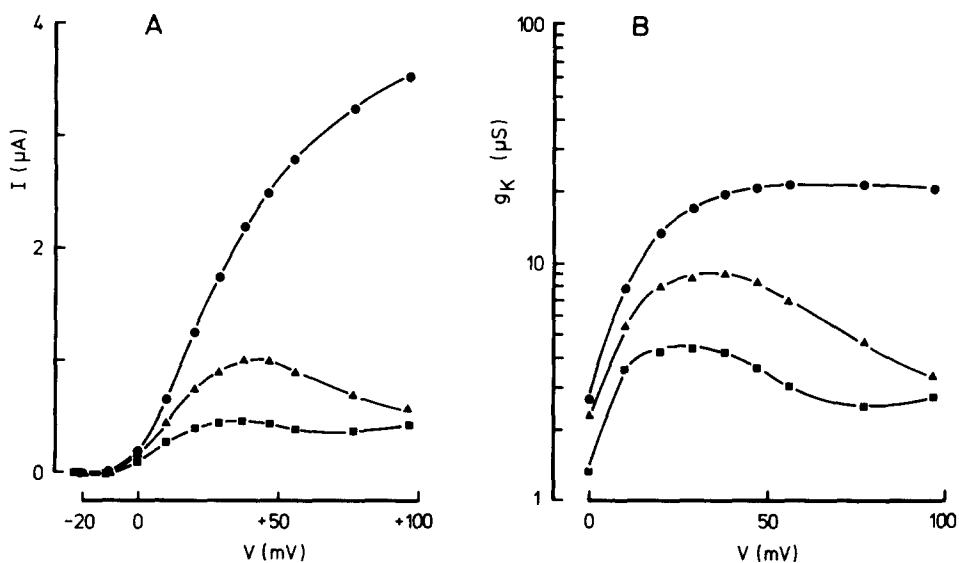


FIGURE 3. Effects of quinidine on the voltage-activated, delayed K^+ outward current. (A) Current-voltage relationship of isochronal currents. Measurements were taken at the end of 160-ms voltage clamp pulses before (circles) and after external application of $50 \mu\text{M}$ quinidine solution for 15 (triangles) and 60 min (squares). The currents were corrected for leakage. The cell was injected with $150 \mu\text{C}$ EGTA. Holding potential, -40 mV . Cell R-15. (B) Conductance-voltage relationship from the same experiment as in A. Conductances were calculated from a potassium equilibrium potential of -75 mV .

Fig. 3 shows current-voltage and conductance-voltage plots of steady state currents before and 15 and 60 min after application of $50 \mu\text{M}$ quinidine. The block is voltage dependent and increases with time after application of the drug. There is almost no block at negative potentials or short times after drug application. At potentials more positive than 30–40 mV, the plots exhibit a region of negative slope. This is consistently observed from conductance-voltage plots, but is not always so clear from the current-voltage plots. Particularly at longer times after drug application (30–60 min), the curves increase at high potentials, which may indicate some relief of the block.

Recovery from the quinidine effects after washout of the drug was poor. Washout of a 100 μM quinidine solution produced only an 8–14% recovery after 30 min.

Time Course of the Block

The block of the delayed K^+ current by quinidine increases with time after application. Fig. 4A shows a normalized plot of isochronal outward currents ($I_{\text{K,Q}}/I_{\text{K}}$) vs. time after application of various concentrations of the drug (I_{K} and $I_{\text{K,Q}}$ are the currents in control solution and after drug application, respectively). There is a fast and a slow phase of the block. The fast phase occurs within seconds after the onset of perfusion. This is shown more clearly in Fig. 4B. The

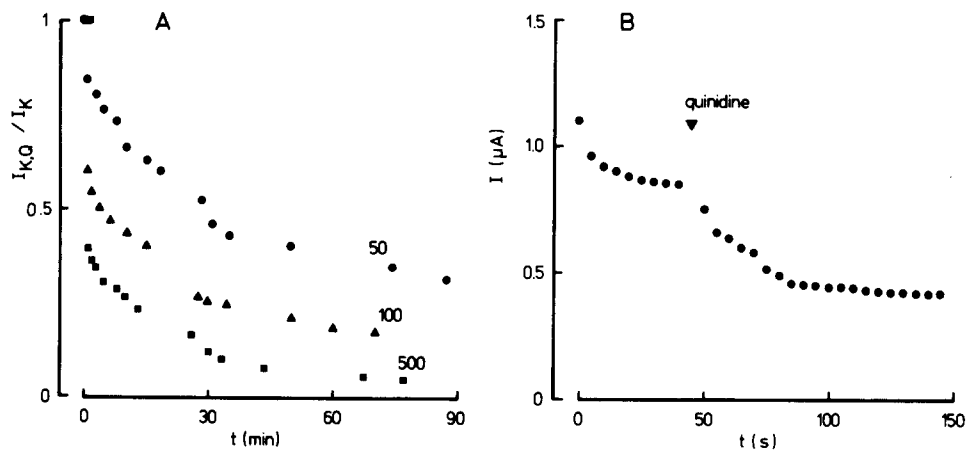


FIGURE 4. Time dependence of the action of quinidine on the voltage-activated, delayed outward K^+ current. (A) Normalized, isochronal currents ($I_{\text{K,Q}}/I_{\text{K}}$) measured at 160 ms were plotted after applications of 50 (circles), 100 (triangles), and 500 μM (squares) quinidine vs. time. Voltage clamp pulses were from -40 to $+28$ mV. The cells (all R-15) were injected with EGTA (150–225 μC). The data were corrected for leakage. (B) Time course of onset of quinidine block. Plot of isochronal K^+ currents elicited by repetitive pulses at 0.2 Hz. The time at which external quinidine (0.5 mM) was introduced is indicated above the response (arrowhead).

cell was stimulated repetitively at 0.2 Hz to obtain a steady state of outward currents. Perfusion of 500 μM quinidine produces an onset of the block within at most 10 s. After the fast phase of the block, a slow phase of decline of currents continues. The slow phase of the block develops approximately exponentially with time constants of 29 min in 50 μM quinidine, 23 min in 100 μM , and 22 min in 500 μM . Both phases, the fast block (current measured 1 min after onset of quinidine-containing solution) and the maximum block (current measured 60 min after the onset of perfusion), are concentration dependent.

Stoichiometry of the Block

The order of the reaction between quinidine and a presumed blocking site was estimated from dose-response curves. Fig. 5 shows a plot of the ratio of outward currents taken at the end of 160-ms pulses, before (I_K) and after 30 min application of quinidine ($I_{K,Q}$) vs. external quinidine concentration. After 30 min in quinidine solution, the block was almost at a maximum in most cases. The experimental data could be fitted by assuming a 1:1 drug-receptor interaction

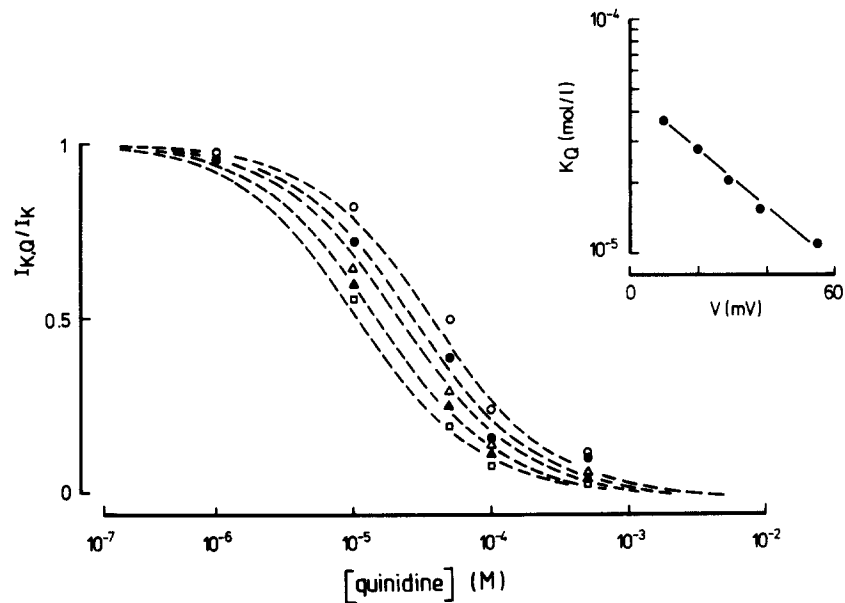


FIGURE 5. Effect of quinidine concentration and membrane potential on the voltage-activated, delayed K^+ outward current. The plot shows dose-response curves of the current ratios $I_{K,Q}/I_K$ vs. the logarithm of the external quinidine concentration at the different test pulse potentials indicated. The stippled lines drawn through the experimental points were calculated after the equation: $I_{K,Q}/I_K = K_{Q,V}/K_{Q,V} + [Q]$, where I_K and $I_{K,Q}$ are the K^+ currents in control solution and after application of quinidine, respectively, $K_{Q,V}$ is the voltage-dependent apparent dissociation constant, and $[Q]$ is the external quinidine concentration. The inset shows a semilogarithmic plot of the apparent dissociation constants as a function of membrane voltage. All data are from cell R-15. $V = +10$ (○), $+20$ (●), $+29$ (△), $+38$ (▲), and $+55$ (□) mV.

and an apparent dissociation constant of $28 \mu\text{M}$ at $V = +20$ mV. More positive membrane potentials shift the dissociation constants to the left on the concentration axis. The inset in Fig. 5 shows values of K_Q plotted on a semilogarithmic scale against membrane voltage. K_Q changes e-fold for a 34-mV change in membrane voltage. From the potential dependence of K_Q , it can be estimated that the drug molecule is sensitive to 74% of the transmembrane electrical field.

Estimation of the Rate Constants of the Block

The outward current in quinidine solution decays during maintained depolarization. The rate of this decay of current is exponential and increases with drug concentration, membrane potential, and time after drug application. At low doses of quinidine ($\leq 10 \mu\text{M}$), there is almost no decay of the outward current at various potentials (measured up to $+80 \text{ mV}$), although the maximum current is reduced. An increase of the decay rate of the current is clearly visible at $50 \mu\text{M}$ quinidine in the bathing solution. Since the stoichiometry of the block is one to one, the rate constants of a reaction



where Q is quinidine and R is the receptor site, can be calculated from $\tau = 1/(k_1[\text{Q}] + k_{-1})$ and $1 - I_{\text{K,Q}}/I_{\text{K}} = k_1[\text{Q}]/(k_1[\text{Q}] + k_{-1})$, where τ is the time constant of the decay of the current, $k_1[\text{Q}]$ and k_{-1} are the pseudo-first-order forward and first-order backward rate constants, respectively, $[\text{Q}]$ is the quinidine concentration, and $1 - I_{\text{K,Q}}/I_{\text{K}}$ is the fraction of blocked channels at equilibrium (if $I_{\text{K,Q}}/I_{\text{K}}$ is the fraction of unblocked channels). The dissociation constant is given by

$$K_{\text{Q}} = k_{-1}/k_1 = [\text{Q}]I_{\text{K,Q}}/I_{\text{K}} - I_{\text{K,Q}}. \quad (2)$$

From these equations, the pseudo-first-order forward rate constant is calculated from

$$k_1[\text{Q}] = (1 - I_{\text{K,Q}}/I_{\text{K}}) \frac{1}{\tau}, \quad (3)$$

and the backward rate constant from

$$k_{-1} = \frac{I_{\text{K,Q}}}{I_{\text{K}}} \frac{1}{\tau}. \quad (4)$$

In Fig. 6 the values for $k_1[\text{Q}]$, k_{-1} , and K_{Q} were plotted vs. membrane voltage. Values of the rate constants and the dissociation constant increase or decrease exponentially with positive membrane potentials. They further change with time after application of the drug, although this appears not to be the case for the backward rate constant. This can be explained by assuming that the drug diffuses into the cell, and with increasing internal quinidine concentration the block occurs faster and comprises more channels. Thus it appears that the equilibrium constants change with the concentration of the drug diffusing in. The data further support the proposed reaction scheme of a concentration-dependent pseudo-first-order forward rate constant and a concentration-independent first-order backward rate constant. The voltage dependence of the rate constants and the voltage dependence of the dissociation constants is not much changed with time after drug application. This further indicates that the mechanism of action does not change with time after application of the drug.

The voltage dependence of the dissociation constant implies that the drug

molecule is sensitive to 68% of the membrane electric field (69 and 72% in two other cells) and compares favorably with the estimate obtained from dose-response plots (74%; see above).

Effects of Increased External K^+ Concentration

A rise in the external K^+ concentration antagonizes the blocking effect of a number of drugs or ions (Armstrong, 1971, 1975; Shapiro, 1977; Eaton and Brodwick, 1980; Armstrong and Taylor, 1980), possibly by competition of K ions with drug molecules. We have looked for a similar phenomenon in our experiments. Raising the external K^+ concentration from normal, 10 mM, to

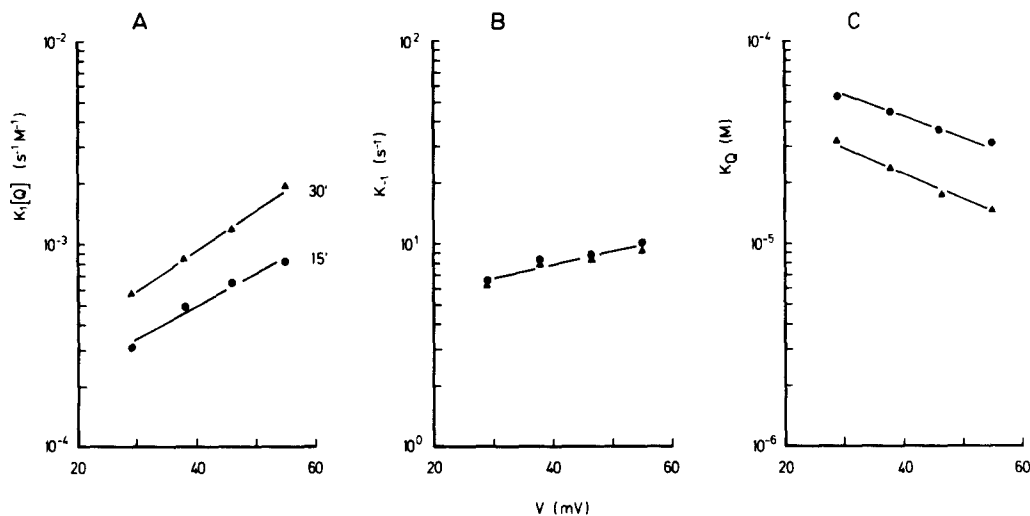


FIGURE 6. Voltage dependence of the rate constants and the dissociation constants of the block produced by external quinidine. Semilogarithmic plots of (A) forward rate constants, (B) backward rate constants, and (C) dissociation constants at 15 (circles) and 30 min (triangles) after application of quinidine ($50 \mu\text{M}$) solution vs. membrane potential. In A, there is an e-fold change of $k_1[\text{Q}]$ per 27 and 21 mV change in potential after 15 and 30 min, respectively; in B, k_{-1} changes e-fold per 65 mV change in potential; and in C, K_Q changes e-fold per 40 and 37 mV change in potential after 15 and 30 min in quinidine solution. The lines drawn to the points are least-square linear regression lines. For further details see text.

100 mM after the cell had been bathed for 15 min in 50 or 100 μM quinidine solution caused only a weak relief of outward currents. In the two cells the peak outward current increased by 5 and 2% and isochronal currents (at 160 ms) increased by 8 and 4% after 25 min in high K^+ . This indicates that the binding of quinidine must be rather strong or the binding site is not easily accessible to external K ions.

pH Effect

At an external pH of 8.9, where the neutral form is favored (67% neutral) compared with pH 7.6 (9% neutral), the K^+ outward current, after it had

obtained a maximum block at pH 7.6, was further reduced by 15%. If the solution with a pH of 7.6 was changed to a solution with a pH of 6.9 (2% neutral), no change of the amount of the block was observed. The same result was obtained with a larger pH jump from 8.9 to 6.9.

Quinidine-4-AP Interaction

Quinidine and 4-AP both block the voltage-dependent, delayed K^+ outward current, but these drugs have a different effect on current inactivation kinetics. While quinidine produces a more rapid inactivation, 4-AP slows the inactivation (Hermann and Gorman, 1981a). Therefore, it appeared interesting to investigate the interaction of these drugs.

Fig. 7 shows the delayed K^+ current in control solution, after application of 2

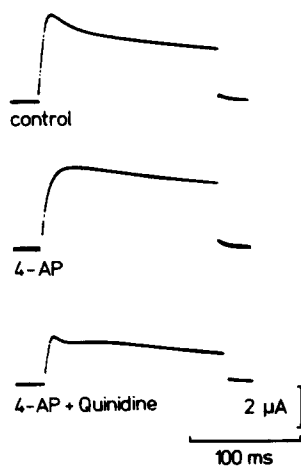


FIGURE 7. Action of quinidine and 4-AP on the voltage-activated, delayed K^+ outward current. Current before (top trace) and after application of 4-AP (2 mM) for 20 min (middle trace), and after application of 4-AP (2 mM) and quinidine (500 μ M) for 15 min (bottom trace). Test pulses were applied from a holding potential of -40 mV to $+98$ mV. Cell L-6.

mM 4-AP and after application of 2 mM 4-AP plus 0.5 mM quinidine. In 4-AP solution the peak current is somewhat reduced and its activation and inactivation kinetics are slowed compared with the current in control solution (cf. Hermann and Gorman, 1981a). If quinidine was in the perfusate in addition to 4-AP, the activation phase was not altered, but its inactivation showed two phases: an early inactivation phase similar to the fast current decay seen in quinidine solution, and a slow inactivation phase that resembles the current waveform seen in 4-AP solution. A similar current waveform was obtained if both drugs were applied simultaneously. The data suggest that the drug molecules compete for binding sites at the K^+ channels.

Internal Effects of Quinidine

To assess whether quinidine acts at the internal side of the membrane, the drug was injected into the cells. Injections were made ionophoretically or by pressure.

Using either method, internal quinidine produced a block of the K^+ current that was essentially similar, as if it had been applied in the external milieu. The peak outward current was reduced and the block increased exponentially during a maintained depolarizing voltage step. The current-voltage and conductance-voltage relationships exhibit a negative slope at positive membrane potentials and the block was concentration dependent. In contrast to the slow time course after external application, the block was maximal 1–2 min after injection of the drug. We do not know the internal concentration of quinidine as we have no measure of the electrode transport numbers for quinidine. Assuming transport numbers of 0.1–0.3, the internal quinidine concentration may have been ~80–250 μM .

Effects of Quinidine on the Fast K^+ Outward Current

The fast K^+ outward current was studied in a solution containing Co^{2+} , TTX, and zero Ca^{2+} at a holding potential of -80 mV. Under these conditions it is possible to selectively activate the fast K^+ current component in a potential range from about -50 to -20 mV. At a holding potential of -40 mV, the current is completely inactivated. After perfusion with quinidine solution (50–500 μM), peak currents and steady state currents were reduced in a dose-dependent, voltage-dependent, and time-dependent manner. A 50% blockade of the fast outward current at $V = -20$ mV occurred at a concentration of 120 μM quinidine. The block increased with more positive membrane potentials. The time course of the block was slow, with 50% inhibition at 19–27 min.

Action of Quinidine on the Ca^{2+} -activated K^+ Current

The Ca^{2+} -activated K^+ current was induced by intracellular, ionophoretic injection of Ca^{2+} as reported previously (Gorman and Hermann, 1979). In Fig. 8B peak outward currents were plotted vs. different membrane holding potentials before (circles) and after external application of 50 μM (triangles) and 500 μM (squares) quinidine. The outward currents were reduced in a dose-dependent manner with a 50% block at $1,400 \pm 160$ μM quinidine. The block almost reaches steady state after 1 min. A further increase of the block of 10% occurred within 15–30 min. The time course of the block was examined in experiments where Ca ions were injected until the outward current obtained a plateau, at which time the bathing medium was rapidly exchanged by quinidine-containing solution (Fig. 8A). Perfusion with 5 mM quinidine caused a 95% block within 22 s. At 1 mM quinidine, the block was 90% complete after 50 s. (The solution exchange in our experimental situation is 95% complete within ~5 s; see Hermann and Gorman [1981a]). Recovery from the block after 5 min in 1 mM quinidine solution was 30% within 1 min. In a different experiment, recovery from the block was 35% after 15 min. The block is voltage dependent and increases with positive membrane potentials. There was no apparent block of the K^+ current at potentials negative to -50 mV. Intracellular injection of quinidine had no blocking effect.

The action of quinidine is complicated by its effect on the decay phase of the Ca^{2+} -activated K^+ current. Fig. 8, C and D, shows that in 2 mM quinidine the

decay phase of K^+ currents is remarkably slowed with time after application. The current responses when normalized and plotted on a semilogarithmic scale vs. time exhibit two phases of decay in control solution (cf. Gorman and Hermann, 1979). At short times after change to quinidine-containing solution (5 min), the fast decay phase is reduced, but its time course is similar to that in control

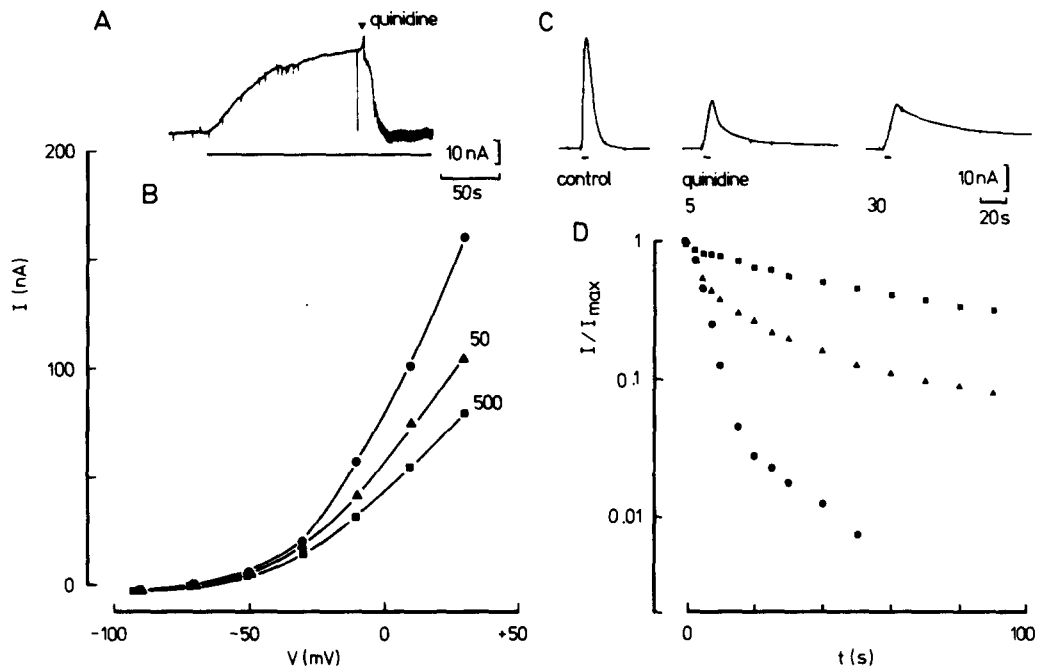


FIGURE 8. Action of quinidine on the Ca^{2+} -activated K^+ current. (A) K^+ current produced by a 150-nA internal Ca^{2+} injection for the time shown by the line beneath the current trace at a holding potential of -30 mV. The time at which external quinidine-containing solution (5 mM) was exchanged is indicated above the response (arrowhead). (B) Current-voltage plot of peak K^+ current activated by intracellular injection of Ca ions ($0.25 \mu C$) at various holding potentials before (closed circles) and after 50 (closed triangles) and 500 μM (closed squares) quinidine for 15 min. A and B are from cells L-6. (C) K^+ outward currents activated by 80-nA internal Ca^{2+} injections for 5 s (bars) at a holding potential of -30 mV before (control) and after application of quinidine (2 mM) for 5 and 30 min. (D) The semilogarithmic plot shows the decay phases of the normalized K^+ currents shown above in C vs. time. Cell R-15. Cells were kept in 10 mM Co^{2+} , 50 μM TTX, and Ca^{2+} -free ASW solution.

solution, while the fraction of the slower decay phase is increased and slowed compared with control. This process proceeds with time (see the 30-min response). After ~ 10 min in quinidine solution, the outward current no longer returned to its previous holding current level.

Because of this effect on the decay phase, estimates of the apparent dissociation

constant from dose-response relations could be in error, especially at high concentrations and long times after drug application. This problem was circumvented to some extent by the following type of experiment. To keep the Ca^{2+} background concentration constant, we injected the Ca^{2+} -chelating agent EGTA into the cells (usually at 250 nA for 10 min). By this means, the response to a standard Ca^{2+} injection was totally abolished. If the magnitude of the Ca^{2+} injection was increased 5–10-fold, an outward current response of an amplitude similar to that before EGTA injection could be elicited. This method allows us to keep the internal, free Ca^{2+} concentration reasonably constant, in spite of continued Ca^{2+} injection and possible effects of the drug on Ca^{2+} buffering. A 50% reduction of the outward current occurred at $\sim 500 \mu\text{M}$ quinidine and, most remarkably, there was no slowing of the decay of the current in the EGTA-injected cells (Hermann, 1983).

Effects of Quinidine on Ca^{2+} and Na^{2+} Inward Currents

Ca^{2+} inward currents were separated by removing K^+ outward currents with TEA (100 mM), 4-AP (2 mM), and injection of EGTA (75–150 μC) or Cs^+ (300 μC) into the cells. TTX (50 μM) was used to block the fast Na^+ inward current. The block of the Ca^{2+} inward current by quinidine is dose and time dependent. A 50% block of peak inward currents measured at $V = +20$ mV, 30 min after application of the drug, was achieved at $260 \pm 130 \mu\text{M}$ quinidine. The time course of action of quinidine was slow and usually took 30 min or more to develop fully. The activation or inactivation time course of the Ca^{2+} inward current was not altered. At low concentrations of the drug ($< 50 \mu\text{M}$), an increase of Ca^{2+} current (by 2–27% at $V = +20$ mV) occurred. This may be due to some further reduction of an overlapping outward current not previously blocked by the other pharmacological blockers. In addition to a reduction of the peak inward current, there is a shift of the threshold for activation and a shift of the maximum inward current to more negative potentials (Hermann, 1983).

To separate the fast Na^+ inward current, the cells were bathed in Ca^{2+} -free ASW solution containing 10 mM Co^{2+} , 50 mM TEA, and 2 mM 4-AP. Quinidine blocks the Na^+ current in a dose-dependent manner with a half-maximal blockade at 680 μM ($V = +20$ mV). The block slowly develops and obtains a maximum after 20–30 min. Activation or inactivation parameters of the Na^+ current were not altered significantly by the drug.

Action of Quinidine on Membrane Currents in Normal ASW Solution

To investigate whether the separation procedure of membrane currents causes any qualitative differences on quinidine effects, nonseparated membrane currents were studied; i.e., quinidine was applied in normal ASW solution. As expected, both the fast phase and the slow phase of inward current, caused by Na^+ and Ca^{2+} fluxes in R-15 cells (Gorman and Hermann, 1982), were reduced by quinidine (0.5 mM). Similarly, the two components of outward currents seen at more positive voltage steps (to +63 mV), which are the voltage-activated, delayed K^+ current and the Ca^{2+} -activated K^+ current, were reduced in a time-dependent manner in quinidine solution (Fig. 9, top records). The effect of

quinidine on the total outward current in normal ASW solution is further shown in the current-voltage plot of Fig. 9. The curve in control solution shows a maximum at about +50 mV and a local minimum at about +100 mV, which is characteristic for these cells (Meech, 1976; Gorman and Hermann, 1979, 1982). After external application of quinidine for 15 min, both the maximum and the minimum of the curve were reduced. The maximum was further reduced after

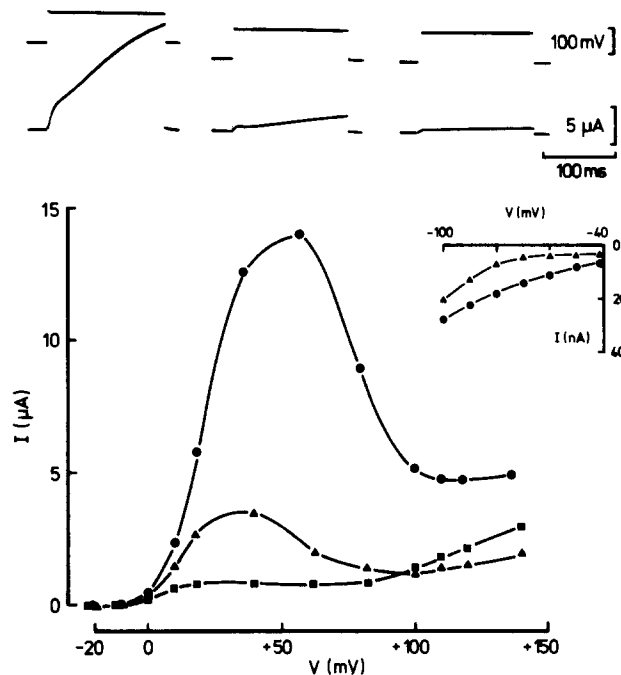


FIGURE 9. Effects of external quinidine on outward and inward currents of cell R-15 in normal ASW solution. Top records: outward currents activated by 160-ms voltage steps to +63 mV before and after 15 and 30 min in ASW solution containing 500 μM quinidine. The plot below shows outward currents measured at the end of 160-ms pulses vs. different membrane potentials before (circles) and after 15 (triangles) and 30 min (squares) in 500 μM quinidine solution. Leakage currents were subtracted from the current records. The inset shows a plot of inward currents in response to hyperpolarizing voltages from the same cell. The currents were measured at the end of 160-ms voltage steps to different negative potentials before (circles) and after (triangles) 30 min of application of 500 μM quinidine. The holding potential was -40 mV in all cases.

30 min in quinidine solution, but the currents at potentials more positive than +100 mV were increased.

Quinidine Effects on Leakage Current and Holding Current

Leakage currents were estimated from the slope of current-voltage plots obtained from steady state measurements of currents produced by hyperpolarizing pulses

from the holding potential. The inset of Fig. 9 shows a plot of steady state currents at negative potentials. In normal ASW solution, quinidine produced an increase of anomalous rectification in the current-voltage curve. At potentials in the vicinity of the holding potential (-30 to -80 mV), the currents were decreased by 20% in 50 μM quinidine and by 80% in 500 μM quinidine. Under zero Ca^{2+} , Co^{2+} , and TTX conditions the effect of external quinidine on membrane leakage was less clear. But in most cases the leakage current was somewhat decreased by 5–30%. If, in addition, EGTA was injected into the cells, however, either no change of the leakage current occurred or, if the leakage current was high, quinidine caused a decrease of this current component. The application of external quinidine (50–500 μM) caused a small outward current (2–10 nA) at a holding potential of -40 mV that slowly developed over 5–20 min. In EGTA-injected cells no such outward current occurred.

Taking these observations together, it appears that quinidine decreases membrane leakage, but also may interfere with a Ca^{2+} -controlled current component

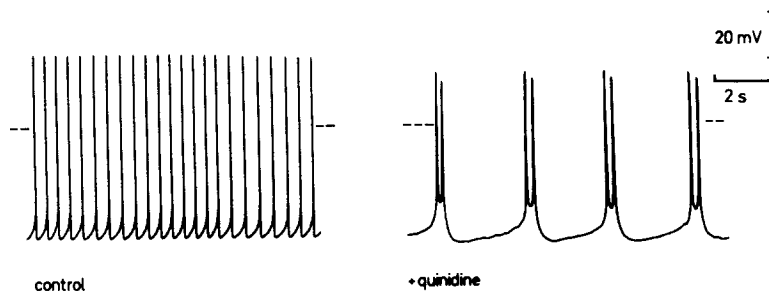


FIGURE 10. Effects of external quinidine on spontaneous discharge activity. Quinidine (500 μM) converts the beating discharge activity of cell L-11 into slow oscillation bursting activity. Conversion to bursting appeared 15 min after drug application. The broken line indicates zero voltage.

or with Ca^{2+} sequestration, which may lead to an increase of the leakage conductance in some cells.

Action of Quinidine on Spontaneous Discharge Activity

Quinidine prolongs the duration of single action potentials and reduces their rate of rise, the peak amplitude, and the afterhyperpolarization. The overshoot was reduced by 30 and 38% in a 500 μM external quinidine solution after 30 min application in two experiments.

In addition to these effects on the parameters of the action potential, quinidine converts a repetitively beating cell into a spontaneously bursting cell (Fig. 10). The bursting discharge activity quickly ceases if external Ca^{2+} is removed, and bursting is restored if Ca^{2+} is added again to the external solution (Hermann, 1983). Bursting is converted back to beating if internal Ca^{2+} accumulation is prevented by injection of EGTA into the cell (not shown), which suggests that Ca^{2+} plays a pivotal role in the drug-induced membrane potential oscillations.

DISCUSSION

Quinidine Effects on Outward Currents

A major finding of this report is that quinidine blocks the voltage-activated, delayed K^+ outward current more effectively than other ionic currents at the molluscan neuron soma membrane and confirms and extends previous work on giant axons (Yeh and Narahashi, 1976; Wong, 1981). Quinidine is a powerful blocker of the delayed K^+ conductance if compared with the traditional K^+ channel blockers. TEA and 4-AP have apparent dissociation constants of 6.0 and 1.5 mM, respectively (Hermann and Gorman, 1981*a, b*), if compared at $V = +20$ mV. Quinidine, with a K_D of 28 μ M, is about 200-fold more effective than TEA and is about 50-fold more effective than 4-AP at the same cells and at the same membrane potential. However, quinidine is less selective as a K^+ channel blocker than these compounds.

The block of the delayed K^+ current by quinidine resembles the block produced by internal quaternary ammonium ions, such as the TEA derivatives, tri-*N*-ethylammonium-R, where R stands for various lengths of the hydrocarbon chain (Armstrong, 1971, 1975; Swenson, 1981), or the larger tetra-*N*-alkylammonium ions (French and Shoukimas, 1981). However, other substances, such as strychnine (Shapiro, 1977), flurazepam (Swenson, 1982), or the divalent cation Ba^{2+} (Eaton and Brodwick, 1980; Armstrong and Taylor, 1980), also appear to fit into this class of molecules or ions with a similar effect on the delayed K^+ outward current. These substances appear to penetrate into K^+ pores that can be occupied either by K ions or by a blocking particle. Opening and closing of the channel can be seen to proceed over a sequence of processes, commencing in a voltage-dependent manner from a closed to an open state and further from an open blocked to a closed blocked state (Armstrong, 1975). The model predicts a concentration-, voltage-, and time-dependent block of K^+ channels. It predicts the decrease of the peak current, the decrease of the equilibrium current, and the increase of the block during maintained depolarization, while the initial rate of rise is not altered. It further predicts the concentration-dependent increase of the blocking rate and the decrease of the dissociation constant. Armstrong and Hille (1972) have shown that for low concentrations of quaternary ammonium ions or small depolarizing pulses, the K^+ currents do not exhibit a transient peak but approach the steady state monotonically, which is similar to the effect of quinidine. The model also accounts for the transient increase in tail currents, which suggests that K^+ channels have to be unblocked before they close. The slowing of the decline of tail currents suggests that the transition from the blocked to the open channel may be rate limiting. A "closed blocked" state of the channels appears to be indicated from our result of a tonic block, which might truly be a closed blocked state with extreme stability. This is also indicated by the multiphase K^+ currents seen at very positive membrane potentials.

The similarity to the TEA derivatives is further obvious considering the physicochemical properties of the quinidine molecule. The extension of the hydrophobic quinoline ring from C_7' to C_2' (see Fig. 1) is ~ 9.6 Å (calculated from X-ray structure determinations; see Wheatley, 1972); this is somewhat

larger than the internal K^+ channel mouth, which has a diameter of $\sim 8 \text{ \AA}$ (Armstrong, 1975; Hille, 1975; Hermann and Gorman, 1981*b*). To fit into the channel mouth, the molecule may therefore have to move into the membrane, where it attaches to a hydrophobic moiety; or the K^+ channel may be larger than previously thought (French and Shoukimas, 1981; Swenson, 1981; Coronado and Miller, 1982). The voltage dependence of the block produced by quinidine indicates that the molecule penetrates 70–80% into the membrane, which supports the notion that K^+ channels in the soma membrane of *Aplysia* neurons have a large nonselective inner mouth extending about three-quarters into the membrane. The distance between C_8 and C_2 of the more hydrophilic quinuclidine ring is $\sim 3 \text{ \AA}$ and is therefore suited to fit into the channel pore, which has a diameter of 3–3.3 \AA (Hille, 1975).

Quinidine may act on both sides of the membrane. An external site is indicated by the fast initial blocking effect within seconds after onset of drug application. This does not seem surprising, as these K^+ channels also have an external receptor site for TEA (Hermann and Gorman, 1981*b*). The existence of an external binding site for quinidine could be challenged, however, as we do not know how fast the molecules can penetrate into or through the membrane to occupy an internal site, particularly if high external quinidine concentrations are used. The indication for an internal blocking site is more convincing. There is (*a*) a slow phase of the block with a time constant of $\sim 30 \text{ min}$, which probably reflects the slow penetration of quinidine through the membrane, and (*b*) a maximum effect on K^+ currents obtained within a few minutes after quinidine injections into the cells. The mechanism of action of quinidine appears similar whether applied externally or internally. In both cases the block increased during maintained depolarization, the current-voltage curve exhibited a negative slope at positive potentials, and the voltage dependences of the rate constants were similar. Our findings suggest that extracellular quinidine and intracellular quinidine bind at the same site.

External quinidine exerts a fast blocking action (within seconds) at the Ca^{2+} -activated K^+ current, while internal injection of the drug had no blocking effect, which indicates that quinidine binds to an external site at the surface of the membrane. This is in agreement with the effects of quinine on red blood cells (Reichstein and Rothstein, 1981). A remarkable feature of the action of quinidine is the slowing of the decay phase of the Ca^{2+} -activated K^+ current. This has important implications for the mode of discharge of spontaneously active cells, as the Ca^{2+} -activated K^+ current plays a major role herein (see below and Hermann, 1983). Quinidine may even effectively increase the total amount of K^+ outward current, although it reduces the peak current. The dissociation constant of the quinidine reaction is about threefold higher compared with the K_D of TEA (Hermann and Gorman, 1981*b*). These effects of quinidine could explain the earlier finding that the drug does not block the Ca^{2+} -activated K^+ conductance (Meech, 1976). In addition, the voltage sensitivity of the drug action may help to explain why the drug is less effective at negative potentials than at positive potentials.

The fast K^+ outward current is also blocked by external or internal quinidine.

The effect of the drug on this current component is less obvious, as it can be studied in isolation only at negative potentials. The K_D at $V = -20$ mV is close to the value expected at that potential for the delayed K^+ current, which suggests that the blocking mechanism may be similar for the two K^+ current components.

Quinidine Effects in Inward Currents

The quinidine concentration needed for a half-maximal block of the Na^+ and the Ca^{2+} inward current is at least an order of magnitude higher than that needed for a block of the delayed K^+ current. The K_D of quinidine needed to block the Na^+ conductance is in a range similar to that reported in rat diaphragm, with a K_D of $184 \mu M$ (Harvey and Rang, 1974), and in squid axon, with a K_D of $240 \mu M$ (Yeh and Narahashi, 1976). These K_D values are more than an order of magnitude higher than those found in frog auricular fibers (Ducouret, 1976) or in isolated ventricular cells (Lee et al., 1981). Na^+ current kinetics were not appreciably affected in *Myxicola* giant axons (Wong, 1981) or in *Aplysia* neurones, but time constants of activation and inactivation were increased in the auricular trabeculae of the frog (Ducouret, 1976).

Suppression of the Ca^{2+} inward current by quinidine as reported here has been also found in frog heart (Ducouret, 1976), and in mammalian heart muscle fibers (Nawrath, 1981), but not in neuroblastoma cells (Fishman and Spector, 1981). A small reduction of the peak Ca^{2+} inward current by quinine was noticed by Plant and Standen (1981) in molluscan neurons, where the drug also appeared to reduce TEA- and 4-AP-resistant outward currents. Our results are in general agreement with these findings. However, we also found that the Ca^{2+} inward current is increased after application of low concentrations of quinidine. In addition, quinidine shifts the current-voltage curve to more negative potentials, which causes a shift in the threshold and results in an increase of inward current at negative potentials. This may have important consequences for the generation of slow membrane potential oscillations that are the basis for bursting discharge activity (see below). The reason for the shift in the current-voltage relation is not known, but it could result from the binding of quinidine to the internal side of the membrane, where it reduces the negative surface potential, or it could reduce the binding affinity of Ca ions.

Quinidine Effects on Spontaneous Discharge Activity

Quinidine and quinine alter the waveform of the action potential in a variety of tissues. The drugs diminish the rate of rise, the overshoot, and the afterhyperpolarization of the action potential and prolong its duration in nerve cells (Yeh and Narahashi, 1976; Fishman and Spector, 1981; Walden and Speckmann, 1981), in cardiac muscle fibers (see Wang and Parker, 1980), and in skeletal muscle (Senges et al., 1973; Andersson, 1973; Harvey and Rang, 1974). We confirm those effects on the action potential in *Aplysia* nerve cells and we are able to explain the effects on basis of the voltage clamp data, which showed that the Na^+ and Ca^{2+} inward currents, as well as various K^+ outward currents, are suppressed by quinidine.

Quinidine, in addition, converts cells that discharge in a regular beating mode

to bursting discharge activity. However, this conversion is difficult to correlate specifically to any of the multiple membrane current effects of the drug. Nevertheless, we are able to offer a plausible explanation for its burst-generating action. The blocking action of quinidine on Na^+ and Ca^{2+} currents is unlikely to produce this effect, since we know from previous experiments that suppression of these currents only modifies repetitive discharge frequency but does not induce bursting (Gorman and Hermann, 1982). A similar effect is expected from the action of the drug on the fast K^+ outward current. The block of the delayed K^+ conductance is also probably not directly involved in the generation of the slow potential oscillations but prolongs action potentials that are primarily Na^+ dependent in the beating L-11 neurons. It therefore appears that the major effect of quinidine in eliciting bursting rhythmogenesis is composed of (a) an increase in Ca^{2+} inward current caused by the shift of the current-voltage relation, which provides for the depolarization during the burst, and (b) an augmentation of K^+ efflux caused by a prolonged decay of the Ca^{2+} -activated K^+ current, which provides for the burst afterhyperpolarization. The decrease of leakage current by quinidine tends to further enlarge any potential fluctuations caused by small current oscillations. This interpretation of the results agrees with and supports the hypothesis of a $\text{Ca}^{2+}/\text{K}^+$ -linked mechanism as the basis for the slow membrane oscillations underlying neuronal bursting pacemaker activity (Gorman et al., 1981, 1982).

We are indebted to Drs. C. M. Armstrong, M. C. Cornwall, and K. Hartung for reading the manuscript and for valuable discussions. We would also like to thank Dr. W. Rathmayer for his support and C. Dittrich for technical assistance.

This work was made possible by a grant from the Deutsche Forschungsgemeinschaft (SFB 156), by U. S. Public Health Service grant NS 11429, and by a Heisenberg grant to A. H.

Received for publication 20 April 1983 and in revised form 3 February 1984.

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