MOLECULAR PHARMACOLOGY, 20:98–106

Quinidine Interactions with *Myxicola* Giant Axons

BRENDAN S. WONG

Laboratory of Biophysics, Intramural Research Program, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20205

Received September 15, 1980; Accepted February 13, 1981

SUMMARY

WONG, B. S. Quinidine interactions with *Myxicola* giant axons. *Mol. Pharmacol.* **20:** 98–106 (1981).

The effects of quinidine, an antiarrhythmic alkaloid, on the membrane conductances of *Myxicola* axonal membrane were investigated by using the voltage-clamp technique. In the presence of 0.2 mM quinidine sulfate, the resting membrane potential was not affected, whereas the repolarization phase of the action potential was prolonged. Under voltage-clamp conditions, potassium currents displayed a distinct peak before decaying exponentially to a plateau level. Both sodium and potassium conductances were suppressed. However, the potassium conductance was suppressed to a much greater extent and displayed a negative slope in the conductance-voltage curve for membrane potentials above +40 mV. The kinetics of sodium currents were not appreciably affected. The block of potassium channels by quinidine displayed both time- and voltage-dependent characteristics. The percentage of reduction in current depended upon the fraction of the potassium channels open under control conditions. Quinidine blocked both outward and inward potassium currents. The block was less in the presence of high external potassium ions. Two-pulse experiments showed that the number of "inactivated" potassium channels was dependent upon the prepulse potential. Recovery from inactivation was enhanced by hyperpolarization. The primary effect of quinidine in *Myxicola* was attributed to a quinidine-induced block of potassium conductance.

INTRODUCTION

Quinidine is the dextrorotatory isomer of the alkaloid quinine. The main pharmacological use of quinidine is in the prevention as well as the treatment of a wide variety of cardiac arrhythmias, ranging from premature beats to atrial fibrillation. It is widely regarded as the drug of choice for converting atrial fibrillation to sinus rhythm. It is generally agreed that quinidine prolongs the refractory period of cardiac muscle by directly altering the electrophysiological properties of the cardiac cell membrane. A variety of theories have been proposed to explain this effect. The majority of information on the possible mechanism of action of quinidine has been obtained by recording transmembrane resting and action potentials with microelectrodes from isolated cardiac muscle preparations (1, 2) and on canine heart *in situ* (3). Its effect on ion fluxes (4) and on contractions of isolated rabbit atria (5) have also been studied. The prolongation of the refractory period has been attributed to the decrease in the slope of depolarization of the membrane action potential (1), to a depression of excitability (6), to changes in the repolarization process (2), to a decrease of sodium influx (4), or to a reduction of potassium efflux (5). Yeh and Narahashi (7) have studied the action of quinidine on squid axonal membrane by utilizing the voltage-clamp technique which provides much better information as to its ionic mechanism of action. The ionic mechanism as well as the physiological and pharmacological characteristics of the *Myxicola* axonal membranes are also well known. This paper extends the work done on squid and presents a more quantitative study on the kinetics of quinidine action on *Myxicola* giant axons. Such data provide valuable information on the mechanism of action of quinidine as well as on the molecular nature of ionic channels in nerve membrane. Knowledge of its mechanism of action on axonal membranes should also be useful in determining the mechanism of action of quinidine in cardiac cells.

METHODS

Myxicola infundibulum were obtained from Maritime Research Associates (Deer Island, N. B., Canada). Methods for preparing and voltage clamping the axon were essentially the same as those described by Binstock and Goldman (8).

The composition of the ASW[1] was: 440 mM NaCl, 10 mM $CaCl_2$, 50 mM $MgCl_2$, and 5 mM tris(hydroxymethyl)aminomethane, pH 7.4. Quinine, quinine hydrochloride, quinidine hydrochloride, and quinidine sulfate

[1] The abbreviations used are: ASW, artificial sea water; TTX, tetrodotoxin; TEA, tetraethylammonium ion.

0026-895X/81/010098-09$02.00/0

were obtained from Sigma Chemical Company (St. Louis, Mo.). Quinidine sulfate was chosen over the other three because of its higher solubility, its practically zero effect on the pH, and its highly reproducible effects. The drug was added directly to ASW and all drug solutions were prepared fresh daily. The concentration of quinidine used in most experiments was 0.2 mM, unless otherwise stated. Higher concentrations gave a faster and more pronounced effect, but also promoted deterioration of the axon. The concentration of 0.2 mM was chosen because it produced a consistent and stable effect within a reasonable period of time, which for *Myxicola* is preferably less than 30 min. This is taken to avoid the effects of natural run-down of the axon. Sodium currents in *Myxicola*, especially, are known to be labile. The dose response of quinidine is complicated because the effect is dependent on membrane potential. It also varied slightly between individual axons and sometimes considerably between different batches of animals. This variability has not been studied systematically, but is most likely due to differences in the permeability of the drug between individual axons. Therefore, no attempt has been made to quantify the dose response of quinidine in *Myxicola* axonal membrane. Yeh and Narahashi (7) reported an apparent dissociation constant for suppressing potassium conductance of 1.5×10^{-4} M for internal application of quinidine hydrochloride in squid at 0 mV (extrapolated from figure 5 in ref. 7). High-potassium ASW is ASW with all of the NaCl replaced by KCl. Experiments on potassium currents alone were performed in the presence of 10^{-6} M TTX (Sigma Chemical Company) to selectively block sodium currents.

Membrane currents were recorded under voltage clamp directly on-line in digital format in response to a series of 20-mV increment step depolarizations to 160 mV at 15-sec intervals and stored in magnetic tape for later analysis. The holding potential was always at the natural resting potential in each solution and ranged between −70 and −80 mV. Compensated feedback was used to reduce the effects of series resistance. However, a residual series resistance amounting to approximately 5 ohm·cm^2 remained uncompensated. A 100-mV hyperpolarizing prepulse from the holding potential lasting 20 msec was used to remove resting sodium inactivation and to assure uniform initial conditions. Liquid junction potentials were corrected according to the values of Cole and Moore (9) which have been shown to be suitable for *Myxicola* axons (10). Membrane currents were corrected for capacitative transient and leakage by summing the currents of equal depolarizing and hyperpolarizing pulses. However, rectification in the leak current-voltage curve is known to occur in *Myxicola* (11). The correction of leakage by this method, though convenient, may produce an underestimate of I_K inhibition, especially at large depolarization potentials. Membrane currents were first recorded in ASW, and the external solution was changed to one containing the drug. Membrane currents in response to a 160-mV depolarization step were recorded every 2 min until the drug effect had reached a steady state. This usually required 15–20 min. At the concentration used, the effects of quinidine were poorly reversible on return to ASW.

Experiments were carried out at 8 ± 0.5° via the control of a Cambion thermoelectric module.

RESULTS

Effects on resting and action potentials. The resting membrane potential was little affected in the presence of 0.2 mM external quinidine sulfate (−0.5 ± 1.4 mV, n = 10). Under current clamp, the ascending phase of the action potential was not appreciably affected, while the descending phase was increased 3–8 times the normal duration. The prolongation of the action potential proceeded as a function of time after the application of quinidine, and reached equilibrium in approximately 15–20 min. The final action potential in the presence of quinidine is similar to that shown by Yeh and Narahashi (7) in squid. However, the prolongation of the repolarization phase in *Myxicola* is always observed to occur before the decrease in the amplitude of the action potential. The amplitude of the action potential was depressed by approximately 15%. Propagated action potentials were completely blocked when the concentration of quinidine exceeded 3 mM.

Effects on membrane ionic currents and conductances. Quinidine reduced both transient sodium (I_{Na}) and steady-state potassium currents (I_K) but with the effect much more pronounced for I_K. In *Myxicola*, in response to a step depolarization under normal conditions, I_K begins to rise after a short delay, reaches a steady state in a few msec and is maintained for the duration of the depolarization. The steady-state I_K was suppressed in the presence of quinidine. The amount of block is voltage-dependent and ranges from approximately 25% at a membrane potential of −40 mV to about 80% at 100 mV. Furthermore, I_K also displayed a distinct peak (Fig. 1), after which it decayed exponentially to a plateau level with sustained depolarization. This effect is especially pronounced for large depolarization potentials. This decrease in I_K is attributed to a quinidine-induced potassium inactivation. The inactivation, however, is incomplete. The term inactivation is used here in the same context as it was used by Armstrong (12) to describe the

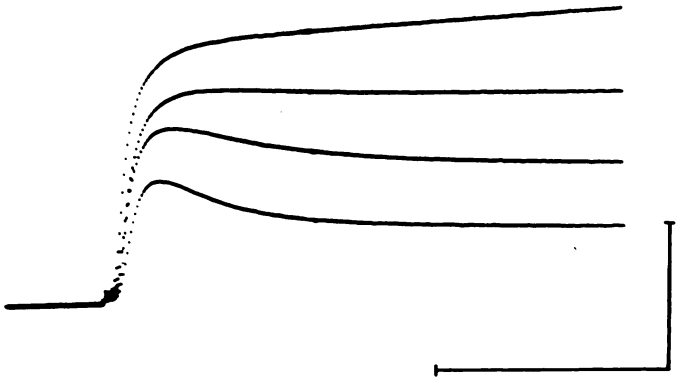

FIG. 1. *Potassium currents in response to a 160-mV step depolarization from rest after application of 0.2 mM quinidine sulfate at 4-min intervals*

The top current trace represents control current in the absence of quinidine. The *vertical* and *horizontal bars* represent 3 mA/cm^2 and 5 msec, respectively.

decay of I_K from a peak value in the squid axon due to the blockage of potassium conductance after injection with quaternary ammonium ion derivatives. This term also implies that the potassium channels have to open first in order for the block to occur. For small depolarizing pulses or for low drug concentrations, no peaks were observed and I_K approached its reduced equilibrium value monotonically. At 0.2 mM, the inactivation of I_K by quinidine becomes more pronounced gradually as a function of time, reaching equilibrium in approximately 15 min, and it does not seem to affect the turn-on kinetics of I_K (Fig. 1).

Sodium currents were obtained by subtracting the currents in the presence of 10^{-6} M TTX from the total currents without TTX. The effect of quinidine on sodium currents is shown in Fig. 2. The presence of small residual steady-state I_{Na} is due to the subtraction of slightly different I_K in the presence and absence of TTX due to the natural run-down of the axon. Although I_{Na} was decreased in the presence of quinidine, the kinetics for sodium activation was not appreciably affected. The effect of quinidine on the time to peak I_{Na} was measured as a function of membrane potential. Values found under the two conditions agreed to within 5%.

At the concentration of quinidine used, no significant effect was observed on leakage currents. However, since leakage tends to increase slightly as time progresses, any small effect caused by quinidine would not be detected.

A typical current-voltage curve is shown in Fig. 3. Leakage conductance had been subtracted from the total current. The current-voltage curve for potassium represents steady-state potassium currents and not peak potassium currents, since the plateau level of I_K is a measure of the steady-state block induced by quinidine. Both inward and outward I_{Na} were partially suppressed, but the reversal potential for the sodium current is unchanged in the presence of quinidine.

Sodium conductance (g_{Na}) and potassium conductance (g_K) were, as defined by Hodgkin and Huxley (13), $g_{Na} = I_{Na}/(V_m - V_{Na})$ and $g_K = I_K/(V_m - V_K)$, where V_{Na} and V_K are the reversal potentials for sodium ion and for potassium ion, and I_{Na} and I_K are the currents for sodium and potassium ions at membrane potential V_m. V_K was assumed to be −80 mV. The sodium conductance was suppressed by approximately 30% in the presence of quinidine and no shift of the curve with membrane potential was observed. The membrane conductance for I_K is plotted in a semilogarithmic scale as a function of membrane potential in Fig. 4. The potassium conductance was appreciably suppressed by quinidine and a negative slope for the conductance curve was observed for membrane potentials above +40 mV. A similar current-voltage curve was obtained by Yeh and Narahashi

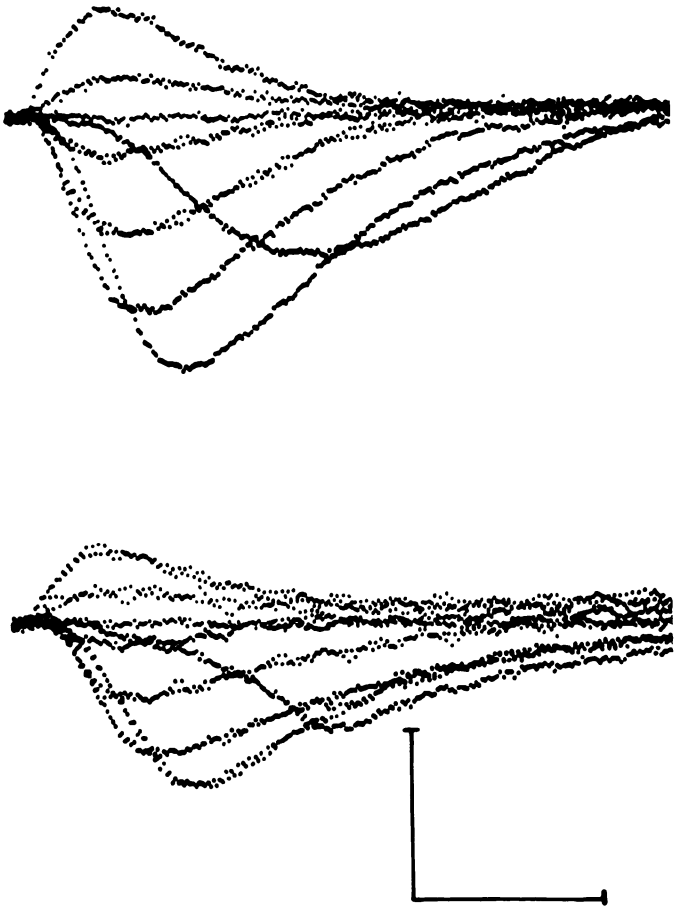

FIG. 2. *Families of voltage-clamped sodium currents from the same Myxicola axon before (top) and after (bottom) application of 0.2 mM quinidine sulfate in response to step depolarizations of 20 mV to 160 mV in increments of 20 mV from the holding potential*

The *vertical* and *horizontal bars* represent 2 mA/cm^2 and 0.5 msec, respectively. Sodium currents were obtained by subtracting the currents in the presence and absence of 10^{-6} M TTX.

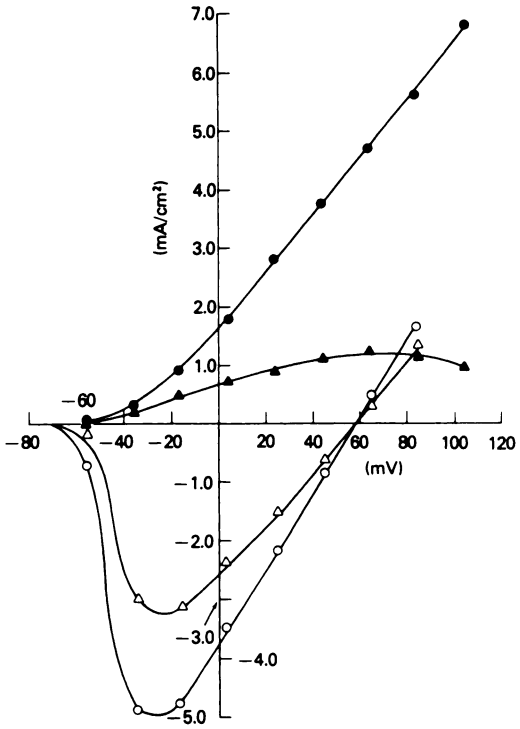

FIG. 3. *Current-voltage curves for peak transient sodium current (I_{Na}) (open symbols) and steady-state potassium current (I_K) (filled symbols) for an intact Myxicola axon before (circles) and after (triangles) application of 0.2 mM quinidine sulfate*

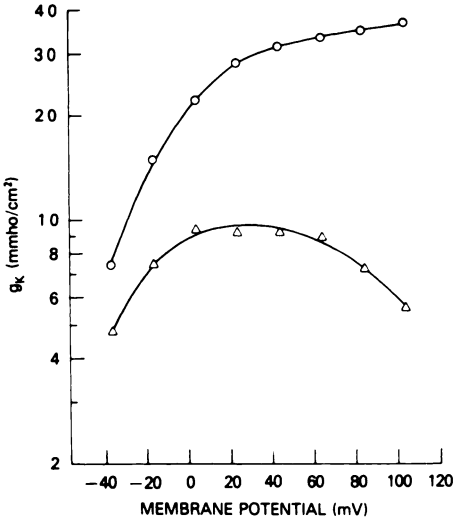

FIG. 4. *Steady-state potassium conductance (g_K) plotted as a function of membrane potential before (circles) and after (triangles) application of 0.2 mM quinidine sulfate*

(7) in squid. However, their I_K represented peak I_K and thus accounts for their not observing a negative slope for the potassium conductance curve in the presence of quinidine.

The effect of quinidine on steady-state sodium inactivation (h_∞) was examined by using the two-pulse method (13). At most, a 1–2 mV shift in the hyperpolarizing direction was observed. That sodium inactivation was not appreciably affected by quinidine can also be seen in the sodium currents shown in Fig. 2.

Assuming that quinidine block obeys first-order kinetics, the ratio (Y_∞) of steady-state I_K in the presence of quinidine to that in ASW represents the fraction of unblocked potassium channels. To demonstrate the voltage dependence of the quinidine block, ($1 - Y_\infty$) is plotted as a function of membrane potential in Fig. 5. The amount of block is increased with a depolarization of the membrane potential, reaching approximately 80% at the highest depolarization used. A similar voltage-dependent block in squid was reported by Yeh and Narahashi (7).

Instantaneous I_K in ASW and high-potassium ASW (440 mM K^+) were obtained by measuring the membrane currents on return of the membrane potential from a 20-msec duration 160-mV depolarizing clamp step to some other value in the presence of 10^{-6} M TTX to block transient sodium currents. The holding potential in high-potassium ASW was +10 mV. Instantaneous currents in ASW were corrected for leakage as described by Binstock and Goldman (10), and those in high-potassium ASW were corrected by using leakage values extrapolated from those obtained in ASW. The effects of quinidine on the instantaneous I_K are shown in Fig. 6. It can be seen that both outward and inward I_K are suppressed. The suppression in ASW, however, is much more pronounced than in high-potassium ASW. At a membrane potential of 70 mV, the suppression in ASW is 74% as compared with 48% in high-potassium ASW.

Inactivation of potassium currents. Two-pulse experiments were performed to confirm that the decrease of I_K in the presence of quinidine is due to inactivation. These experiments were again performed in the presence of 10^{-6} M TTX to block all transient sodium currents. In order to study the development of block induced by quinidine, the prepulse potential was varied and then followed by a constant command pulse of 160-mV depolarization from the holding potential. All prepulses were 20 msec in duration, which is sufficient to attain a steady-state block level. The ratio of peak I_K during the command pulse with a prepulse to that without a prepulse gives an estimate of the fraction of g_K that is not inactivated by the prepulse. The inactivated fraction can then be obtained by subtracting this value from unity. The inactivated fraction as a function of prepulse potential is shown in Fig. 7 and can be seen to be dependent upon the prepulse potential.

A second set of experiments was performed in which the prepulse duration, instead of the prepulse potential, was varied. To study the time development of the block,

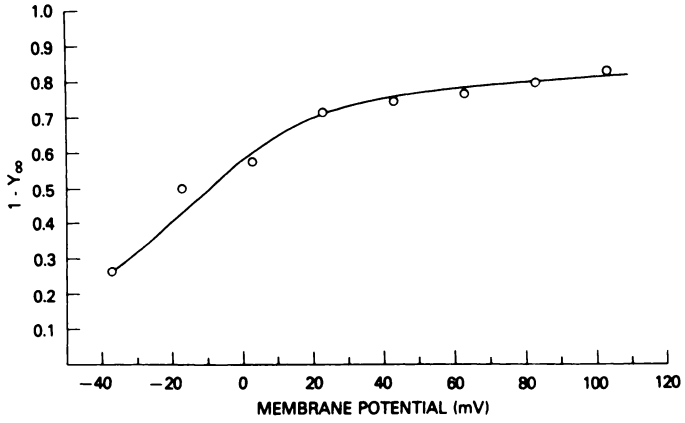

FIG. 5. *Fraction of blocked potassium channels ($1 - Y_\infty$) determined from the ratio of steady-state I_K in 0.2 mM quinidine sulfate to that in ASW plotted as a function of membrane potential*

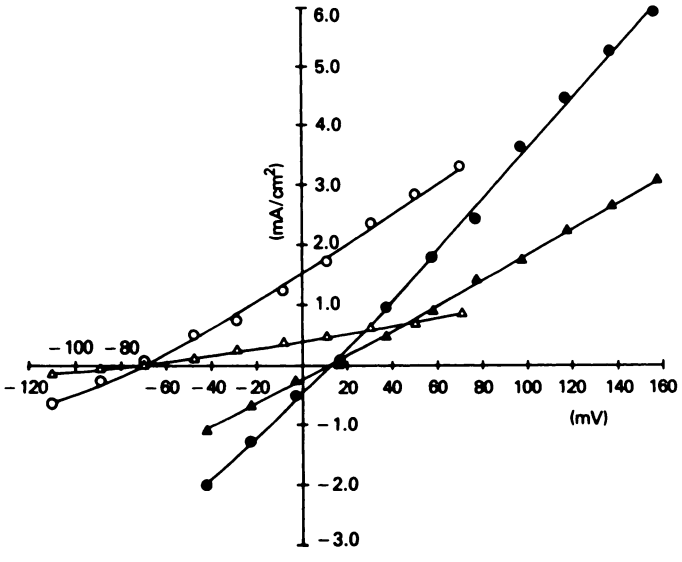

FIG. 6. *Instantaneous I_K before (circles) and after (triangles) application of 0.2 mM quinidine sulfate in ASW (open symbols) in one Myxicola axon, and 440 mM K^+ ASW (filled symbols) in a different axon*

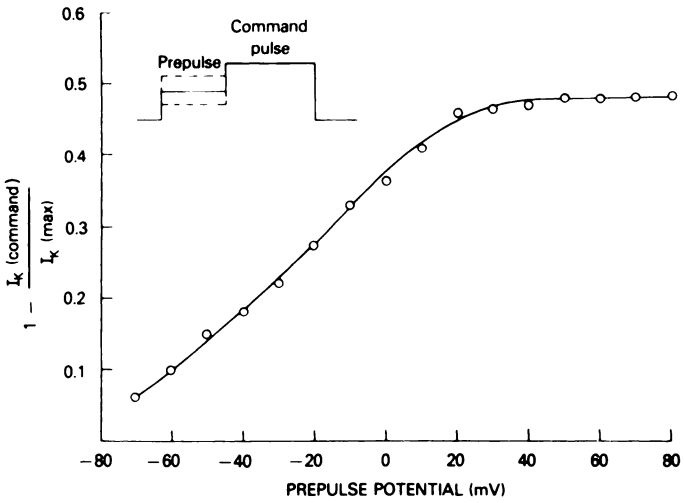

FIG. 7. *Fraction of blocked potassium channels determined from the ratio of peak I_K in 0.2 mM quinidine sulfate plotted as a function of prepulse potential*

Pulse sequence used is shown in *inset*.

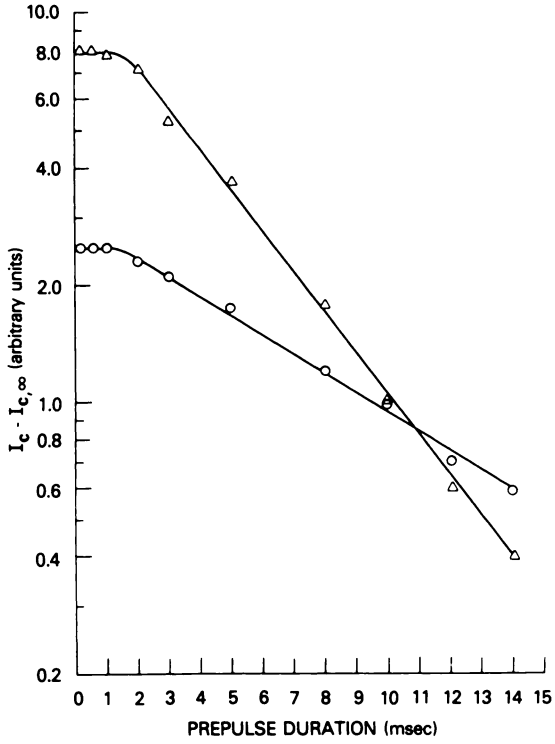

FIG. 8. *Potassium channel block in 0.2 mM quinidine sulfate plotted as a function of prepulse duration for prepulse potentials of −33 mV (circles) and +7 mV (triangles)*

the duration of a small prepulse before the 160-mV depolarization command pulse was varied. The maximum amplitude of the current during the command pulse (I_C) gives an estimate of the fraction of potassium channels that remained unblocked. For long prepulses, the peak command current approached a minimum value ($I_{C,\infty}$). For prepulses shorter than 1 msec, I_C remained essentially unchanged because I_K has not yet turned on. The value, $(I_C - I_{C,\infty})$ for two different prepulse potentials is plotted in a semilogarithmic scale against prepulse duration in Fig. 8. For prepulse durations longer than 2 msec, the block can be seen to develop exponentially. The time constants of the block (time to reach $1/e$ of its initial value) are 4.1 and 8.5 msec, respectively, for prepulse potentials of +7 and −33 mV, with a faster time constant for a more depolarized prepulse. For very large depolarizing prepulse potentials, the time constant of block saturates at around 2.3 msec.

Recovery from I_K inactivation was also monitored by varying the pulse interval between two identical pulses (5 msec in duration and 160 mV depolarized from the holding potential) and observing the ratio of their peak I_K amplitudes. The first pulse is used to open the potassium channels which then inactivate by the action of quinidine. They recover when the membrane potential is repolarized and the second pulse is used to monitor the degree of recovery. The ratio of peak I_K as a function of pulse interval is plotted in Fig. 9. When the first pulse was returned to the resting potential, the time required for 50% recovery was approximately 5 msec. However, when the membrane potential during the pulse interval was held 40 mV hyperpolarized with respect to rest, the time for 50% recovery was less than 3 msec. In both cases, full recovery took approximately 40 msec.

DISCUSSION

The effects of quinidine on ionic conductances were first studied by Yeh and Narahashi (7) on squid axonal membranes. Although quinidine-induced potassium inactivation was also observed in squid, it had not been studied in detail. The present study extends these observations to the *Myxicola* axonal membrane with emphasis on the kinetics of the quinidine-induced potassium inactivation.

The prolongation of the repolarization phase of the action potential can be due to a decrease in the potassium conductance and/or to the slowing or removal of sodium inactivation. From the studies on sodium currents alone, as well as on steady-state sodium inactivation, it can be

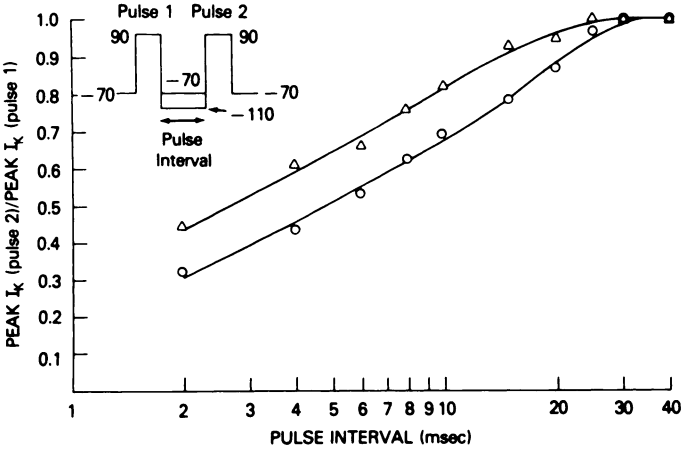

FIG. 9. *Time course of potassium current recovery from inactivation with quinidine*

The pulse sequence used is shown in the *inset*. The ratio of peak I_K from Pulse 2 to that of Pulse 1 is plotted as a function of pulse interval for repolarization potentials at rest (○) and 40 mV hyperpolarized from rest (△).

seen that quinidine does not affect the kinetics of sodium inactivation. Therefore, the prolongation of the repolarization phase of the action potential in *Myxicola* giant axons in the presence of quinidine is due to the effects of I_K inactivation, and thus a depression of steady-state g_K.

The block of potassium channels by quinidine exhibits both voltage- and time-dependent characteristics. Quinidine is proposed to interact with the conductance pathway. It suppresses I_K much more pronouncedly at large depolarizations. The degree of block is dependent on the number of open potassium channels. The kinetics of the gating mechanism is assumed to be unaffected by quinidine. This assumption is supported by the observation that the initial rate of rise of I_K remains unchanged in the presence of quinidine (Fig. 1). It suppresses both outward and inward I_K, and the block is to a lesser extent in high-potassium ASW than in normal ASW. Two-pulse experiments tend to support the theory that the decrease in I_K is due to a drug-induced inactivation.

Several other agents are also known to cause I_K inactivation. Examples are the tropine esters (14), dibucaine (15), certain quaternary amines (12), certain veratrum analogues (16), certain morphine analogues and antagonists (17), sparteine (18, 19), and strychnine (20).

The data presented suggest that the block of potassium channels by quinidine is similar to internal quaternary ammonium ions observed in squid (12) and frog node of Ranvier (21). Armstrong (12) proposes in his model that quaternary ammonium ions bind to a hydrophobic site within the potassium channel. This site is accessible only from the axoplasmic side, but not from the external solution. The blocking ions can only enter a channel whose activation gate is open, and they are propelled by the same diffusional and electrical forces which normally drive potassium ions into the channel. This model predicts quite accurately the changes in I_K found with quaternary ammonium ions inside the squid giant axon. Similarly, the action of quinidine can be explained by the simplest scheme proposed by Armstrong (12) for the action of quaternary ammonium ions:

$$\text{Closed channel} \underset{\text{kinetics}}{\overset{\text{Hodgkin-Huxley}}{\rightleftharpoons}} \text{open channel} \overset{k_{+1}}{\underset{k_{-1}}{\rightleftharpoons}} \text{blocked channel}$$

where k_{+1} is proportional to the axoplasmic concentration of quinidine and k_{-1} is proportional to the reciprocal of the tightness of binding. Armstrong (12) and Armstrong and Hille (21) have also demonstrated that the presence of a peak in I_K depends on the relative values of the normal Hodgkin-Huxley potassium rate constants, α_n and β_n, and the quinidine blocking and unblocking rates, k_{+1} and k_{-1}. The degree of inactivation is related to the tightness of binding of quinidine to the channel.

To differentiate behavior due indirectly to the voltage dependence of gating from that due to characteristics of the blocking reaction itself, the kinetic parameters were analyzed by using computer curve-fit of raw records of I_K. The Hodgkin-Huxley and quinidine-induced inactivation rate constants for a typical *Myxicola* axon were determined in a manner similar to that used by Armstrong (12). Briefly, the maximum potassium conductance, $\bar{g}_K$, was determined from the current and the driving force at a membrane potential V_m = +130 mV by assuming that V_K = −80 mV and that β_n = 0 at this V_m. A $\bar{g}_K$ of 39.3 mmho/cm^2 was obtained. If the assumption is made that the total number of potassium channels (the sum of closed, open, and blocked) is constant, the kinetic scheme can be reduced to two linear first-order differential equations. Values for α_n and β_n were first determined by fitting the currents I_K in a normal axon with $k_{+1} = k_{-1} = 0$. Then, with α_n, β_n, and $\bar{g}_K$ fixed, k_{+1} and k_{-1} were varied to obtain the best fit to I_K in the presence of quinidine and at the same membrane potential. Implicit in the fitting process are the assumptions that α_n, β_n, and $\bar{g}_K$ are not affected by quinidine and that the rate constants can be determined uniquely. The experimental and fitted I_K in the absence and presence of quinidine for a typical *Myxicola* axon are shown in Fig. 10. The corresponding rate constants used to obtain the best fit are shown in Table 1. For the kinetic scheme proposed, the fraction of unblocked channels in the steady state (Y_∞) is equivalent to $k_{-1}/(n_\infty k_{+1} + k_{-1})$, where $n_\infty = \alpha_n/(\alpha_n + \beta_n)$. The experimental and predicted Y_∞ are also shown in Table 1. The good fit obtained suggests that the simple kinetic model proposed is adequate in describing most of

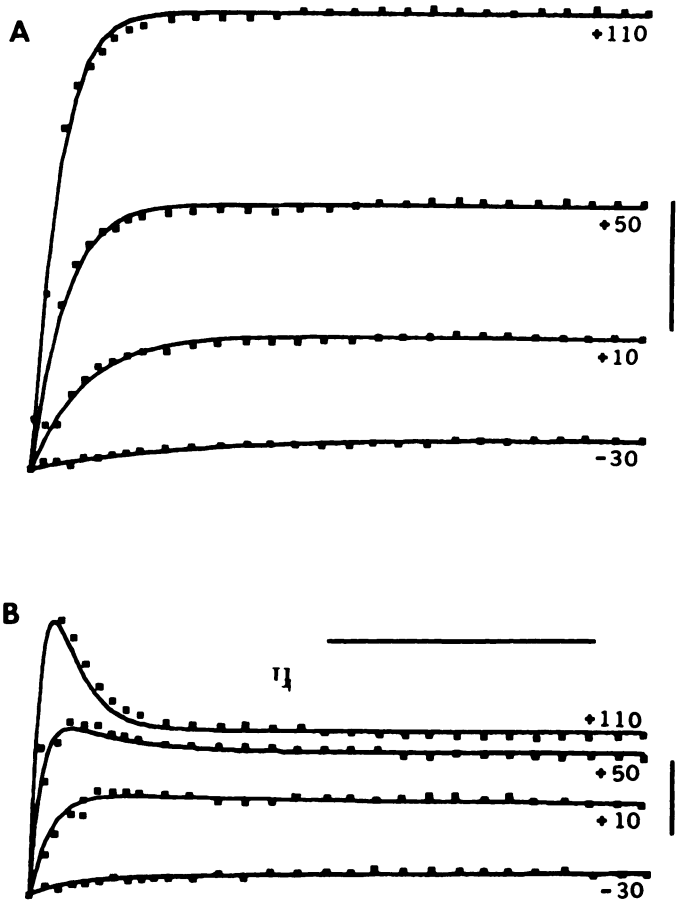

FIG. 10. *Potassium currents before (A) and after (B) the application of 0.2 mM quinidine sulfate in ASW*

■, Experimental data. The continuous line was fitted to the data by using the kinetic model and fitting method described under Discussion. The *vertical* and *horizontal bars* represent 2 mA/cm^2 (top), 0.5 mA/cm^2 (bottom), and 10 msec, respectively. The *numbers* next to each *curve* correspond to the membrane potential.

TABLE 1

Hodgkin-Huxley and quinidine (0.2 mM) blocking and unblocking rate constants for outward potassium currents in a Myxicola giant axon

Membrane potential	α_n	β_n	k_{+1}	k_{-1}	Y_∞	
					Experimental	Predicted
mV		*msec*$^{-1}$				
−30	0.02	0.32	0.24	0.04	0.69	0.73
−10	0.06	0.29	0.85	0.10	0.41	0.41
10	0.15	0.36	0.88	0.13	0.33	0.34
30	0.29	0.38	1.07	0.20	0.29	0.30
50	0.49	0.37	1.68	0.35	0.26	0.27
70	0.69	0.29	1.23	0.27	0.22	0.24
90	0.90	0.17	1.98	0.44	0.19	0.21
110	1.07	0.03	1.91	0.41	0.16	0.18

the experimental data without the need to postulate changes in the kinetics of channel gating. Both k_{+1} and k_{-1} were found to increase only slightly with voltage. Their voltage dependence is approximately 105 mV and 75 mV, respectively, per *e*-fold change. Thus, the binding of quinidine to the open channels is fairly independent of voltage. The observed incomplete inactivation implies the binding of quinidine is relatively loose and is supported by k_{-1} being equal to approximately 0.4 msec^{-1} for large depolarizations.

Although the quinidine-induced inactivation of outward I_K can be accounted for quite satisfactorily by the kinetic model proposed by Armstrong (12), one major difference that cannot be explained and is different from that predicted by the model is that quinidine also blocks inward I_K, whereas TEA leaves inward I_K unaffected. The latter observation is true at least in the squid giant axon. However, it is known from a previous study (22) that *Myxicola* axonal membrane behaves differently from that of squid when subjected to external TEA. While externally applied TEA produces little or no effect in squid, it is found to block fairly specifically, although in a voltage-independent manner, I_K in *Myxicola*. There is no inactivation of I_K and external TEA also blocks both outward and inward I_K when tested in high-potassium ASW. It was postulated (22) that TEA receptors are present on the external surface of the *Myxicola* axonal membrane similar to those reported in the frog node. Thus, the blocking of inward I_K by quinidine can perhaps be explained by the binding of external quinidine to such receptors. However, binding of external TEA (100 mM) to such receptors was found to depolarize the resting membrane potential by approximately 25 mV. That quinidine does not affect the resting potential tends to suggest that it does not act on the same external sites as TEA.

Alternatively, the results can also be explained by the presence of a common blocking site inside the channel. The blocking of inward I_K by quinidine then occurs by a similar mechanism to the block of outward I_K; it is swept into the potassium channel together with the flow of potassium ions and then blocks the channel by being physically too large to pass through. Therefore, quinidine entering either end of the channel will block the corresponding flow of potassium ions, but not the flow in the opposite direction. Thus, the properties of the potassium channel in *Myxicola* may be more symmetrical at its two ends than that in squid, and similar to that found in *Xenopus laevis* nodes (23). Further studies will have to be made to distinguish whether there is a common blocking site or separate sites for internal and external quinidine.

Yeh and Narahashi (7) postulated that, in squid, quinidine acts from the axoplasmic surface in suppressing both $\bar{g}_{Na}$ and $\bar{g}_K$ with the cationic form being the active species for inhibiting $\bar{g}_{Na}$. This theory is supported by the observation that the charged quaternary quinidine methiodide is effective only when applied to the axoplasmic side. In view of the relatively slow action of quinidine to reach a steady state when applied externally (15–20 min), it also seems reasonable to assume that quinidine acts on the internal surface of the *Myxicola* axonal membrane after having penetrated the membrane. Its primarily irreversible effect when the drug is removed from the external solution also tends to support this assumption. The current traces in Fig. 1 which show the peak and steady-state I_K decreasing as a function of time but without affecting the turn-on of I_K in the presence of 0.2 mM quinidine also correlates well with a gradual increase in the axoplasmic concentration of quinidine by diffusion through the membrane. The model proposed by Armstrong predicts that the initial rate of turn-on of I_K should not be affected by an increase in the concentration of the drug, although the rate of blocking should be increased resulting in a decrease of both peak and steady-state currents.

To summarize, the model proposed is that internal quinidine blocks outward I_K, whereas external quinidine blocks inward I_K. In ASW and at potentials depolarized from rest, the net I_K is outward, which implies that external quinidine plays little or no effect in blocking I_K under these conditions. However, one must bear in mind that the proposed model is not unique but to a first approximation gives a reasonable fit of the data.

Two-pulse experiments in which the prepulse duration was varied (Fig. 8) support the postulate that quinidine only blocks open channels. The block develops with a time lag. For times short compared to the time to peak current, no change in peak I_K was observed. Inactivation rate is also dependent upon membrane potential. The time constant of block is faster for large depolarizations, correlating well with their faster rates of activation and the condition that activation must precede inactivation.

The block by quinidine is reversed by repolarization. Hyperpolarization of the membrane potential (with respect to rest) during the interval between the two pulses speeded up recovery (Fig. 9). The faster recovery time can be due to either a decrease in k_{+1} or an increase in k_{-1}. The inward tail currents associated with repolarizations may also help clear channels with blocking ions present. This is different from that observed in frog nodes with TEA derivatives (21), where hyperpolarization increases the time of recovery. The latter effect is probably due to the trapping of the blocking ions in the channel with the closing of the activation gate (21).

The amount of block is decreased in high-potassium ASW (Fig. 6). This may be due to some interaction between potassium ions and quinidine, such as extracel-

lular potassium being capable of displacing quinidine from the blocking site, as well as the differences in the number of open potassium channels because of the different holding potentials used.

In cardiac tissues, the prolongation of the action potential in the presence of quinidine has been suggested by Armitage (5) to be due to a diminished permeability of the cell membrane to potassium ions. This is not unreasonable, since it is known that there is an increase efflux of potassium ions associated with atrial and ventricular fibrillation (24). Radioisotope studies (4) have also shown a depressant effect of quinidine on K^+ efflux and Na^+ influx. These observations are consistent with the data presented in this paper.

From various microelectrode studies of isolated cardiac preparations, it is known that quinidine exerts its antiarrhythmic property by interfering in some way with the permeability of Na^+ and K^+ in the cardiac membrane. However, there has been considerable disagreement as to which of these two effects plays the major role in controlling arrhythmia. Studies previous to West and Amory (2) have all emphasized the effect quinidine has on decreasing the rate of the depolarization process and thus the effect on Na^+ permeability in cardiac muscle. Recently, Hondeghem and Katzung (25) have also emphasize the blocking of sodium channels in cardiac cells as responsible for directly reducing the conduction velocity and the antiarrhythmic action of quinidine. However, West and Amory (2) found that quinidine only depressed the rate of depolarization when the stimulation frequencies were high, but depressed the rate of repolarization at all frequencies of stimulation in isolated rabbit atria.

In this study, although quinidine reduced both I_{Na} and I_K, as long as the action potential is not completely blocked, the effect on I_K is much more pronounced than on I_{Na}. Also, the kinetics of I_{Na} does not seem to be affected. Since the characteristic of a therapeutic concentration of quinidine is a prolongation of the action potential (26), it seems reasonable to suggest that the effect of quinidine on I_K is probably what is primarily responsible for its antiarrhythmic activity.

Recently, quinine has been reported to block calcium-dependent potassium channels in erythrocytes (27) and cultured nerve cells (28). The mechanism of action of quinine in these cells is not known. However, there is no evidence that the voltage-dependent I_K in *Myxicola* and squid axons is also calcium-dependent. Further experiments are needed to determine whether quinine and quinidine have the same or different mechanisms of action in voltage- and calcium-dependent potassium channels.

This paper describes the mechanism of action of quinidine on neural tissue. The technique of voltage-clamping cardiac muscle cells has been plagued with technical difficulties (29), but recent reports using the two-micropipette technique on rabbit Purkinje fibers (30) and on enzyme-dispersed single heart muscle cells (31) have shown great promise and thus provide the opportunity to study the effects of quinidine in cardiac muscle directly by the voltage-clamp technique. The experiments on *Myxicola* help provide clear hypotheses to be confirmed by studies on cardiac tissues.

ACKNOWLEDGMENTS

The author thanks Drs. G. Ehrenstein, M. Huang, H. Lecar, and N. Moran for their thorough reading of the manuscript and their suggestions and Dr. R. Fitzhugh for the use of his computer programs to transfer data to the DEC-10 computer of Division of Computer Research and Technology at the National Institutes of Health for analysis with Modeling Laboratory.

REFERENCES

1. Vaughan Williams, E. M. The mode of action of quinidine on isolated rabbit atria interpreted from intracellular potential records. *Br. J. Pharmacol.* **13:** 276–287 (1958).
2. West, T. C., and D. W. Amory. Single fiber recording of the effects of quinidine at atrial and pacemaker sites in the isolated right atrium of the rabbit. *J. Pharmacol. Exp. Ther.* **130:**183–193 (1960).
3. Prinzmetal, M., K. Ishikawa, H. Oishi, E. Ozkan, J. Wakayama, and J. M. Baines. Effects of quinidine on electrical behavior in cardiac muscle. *J. Pharmacol. Exp. Ther.* **157:**659–664 (1967).
4. Klein, R. L., W. C. Holland, and B. Tinsley. Quinidine and unidirectional cation fluxes in atria. *Circ. Res.* **8:**246–252 (1960).
5. Armitage, A. K. The influence of potassium concentration on the action of quinidine and of some antimalarial substances on cardiac muscle. *Br. J. Pharmacol.* **12:**74–78 (1957).
6. Hoffman, B. F., and P. F. Cranefield. *Electrophysiology of the Heart.* McGraw-Hill, New York (1960).
7. Yeh, J. Z., and T. Narahashi. Mechanism of action of quinidine on squid axon membranes. *J. Pharmacol. Exp. Ther.* **196:**62–70 (1976).
8. Binstock, L., and L. Goldman. Current- and voltage-clamped studies on *Myxicola* giant axons; effect of tetrodotoxin. *J. Gen. Physiol.* **54:**730–740 (1969).
9. Cole, K. S., and J. W. Moore. Liquid junction and membrane potentials of the squid giant axon. *J. Gen. Physiol.* **43:**971–980 (1960).
10. Binstock, L., and L. Goldman. Rectification in instantaneous potassium current-voltage relations in *Myxicola* giant axons. *J. Physiol. (Lond.)* **217:** 517–531 (1971).
11. Goldman, L., and L. Binstock. Leak current rectification in *Myxicola* giant axons: constant field and constant conductance components. *J. Gen. Physiol.* **54:**755–764 (1969).
12. Armstrong, C. M. Inactivation of the potassium conductance and related phenomena caused by quaternary ammonium ion injected in squid axons. *J. Gen. Physiol.* **54:**553–575 (1969).
13. Hodgkin, A. L., and A. F. Huxley. The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*. *J. Physiol. (Lond.)* **116:** 497–506 (1952).
14. Blaustein, M. P. Action of certain tropine esters on voltage-clamped lobster axon. *J. Gen. Physiol.* **51:**309–319 (1968).
15. Narahashi, T., J. W. Moore, and R. N. Poston. Anesthetic blocking of nerve membrane conductance by internal and external applications. *J. Neurobiol.* **1:**3–22 (1969).
16. Ohta, M., T. Narahashi, and R. F. Keeler. Effects of veratrum alkaloids on membrane potential and conductance of squid and crayfish giant axons. *J. Pharmacol. Exp. Ther.* **184:**143–154 (1973).
17. Frazier, D. T., M. Ohta, and T. Narahashi. Nature of the morphine receptor present in the squid axon. *Proc. Soc. Exp. Biol. Med.* **142:**1209–1214 (1973).
18. Ohta, M., and T. Narahashi. Sparteine interaction with nerve membrane potassium conductance. *J. Pharmacol. Exp. Ther.* **187:**47–55 (1973).
19. Schauf, C. L., C. A. Colton, J. S. Colton, and F. A. Davis. Aminopyridines and sparteine as inhibitors of membrane potassium conductance: effects on *Myxicola* giant axons and the lobster neuromuscular junction. *J. Pharmacol. Exp. Ther.* **197:**414–425 (1976).
20. Shapiro, B. I. Effects of strychnine on the potassium conductance of the frog node of Ranvier. *J. Gen. Physiol.* **69:**897–914 (1977).
21. Armstrong, C. M., and B. Hille. The inner quaternary ammonium ion receptor in potassium channels of the node of Ranvier. *J. Gen. Physiol.* **59:**388–400 (1972).
22. Wong, B. S., and L. Binstock. Inhibition of potassium conductance with external tetraethylammonium ion in *Myxicola* giant axons. *Biophys. J.* **32:** 1037–1042 (1980).
23. Koppenhöfer, E. Die Wirkung von Tetraäthylammoniumchlorid aud die Membranströme Ranvierscher Schnürringe von *Xenopus laevis*. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere* **293:**34–55 (1967).
24. Armitage, A. K., J. H. Burn, and A. J. Gunning. Ventricular fibrillation and ion transport. *Circ. Res.* **5:**98–104 (1957).
25. Hondeghem, L. M., and B. G. Katzung. Time- and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. *Biochim. Biophys. Acta* **472:**373–398 (1977).
26. Conn, H. L. Some consideration of quinidine and procaine amide action at the cellular level, in *The Myocardial Cell*, (S. A. Briller and H. L. Conn, eds.). Univ. of Penn. Press, Philadelphia, 269–296 (1966).

27. Armando-Hardy, M., J. C. Ellory, H. G. Ferreira, S. Fleminger, and V. L. Lew. Inhibition of the calcium induced increase in the potassium permeability of human red blood cells by quinine. *J. Physiol. (Lond.)* **250**:32P (1975).
28. Fishman, M. C., and I. Spector. Blockage of a calcium-dependent potassium conductance in neuroblastoma cells by quinine. *J. Supramol. Struct.* (suppl.) **4**:83 (1980).
29. Johnson, E. A., and M. Lieberman. Heart: excitation and contraction. *Annu. Rev. Physiol.* **33**:479–532 (1971).
30. Colatsky, T. J., and R. W. Tsien. Sodium channels in rabbit cardiac Purkinje fibres. *Nature (Lond.)* **278**:265–268 (1979).
31. Lee, K. S., T. A. Weeks, R. L. Kao, N. Akaike, and A. M. Brown. Sodium current in single heart muscle cells. *Nature (Lond.)* **278**:269–271 (1979).

Send reprint requests to: Dr. Brendan Wong, Laboratory of Biophysics, Building 36, Room 2A29, National Institutes of Health, Bethesda, Md. 20205.