Br. J. Pharmacol. (1991), **103**, 1807–1813

Quinidine-induced inhibition of the fast transient outward K^+ current in rat melanotrophs

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1 The effect of quinidine on the fast-activating, fast-inactivating potassium current (I_K(f)) in acutely dissociated melanotrophs of the adult rat pituitary was examined. Macroscopic currents were measured by use of the whole-cell configuration of the patch clamp technique.

2 Bath application of quinidine caused a dose-dependent reduction of the peak amplitude of I_K(f). The K_d for blockade of I_K(f) at 0 mV was estimated to be 41 ± 5.6 μM.

3 Quinidine elicited a dose-dependent increase of the rate of the decay of I_K(f) and this effect was enhanced by membrane depolarization. The possibility that this phenomenon reflects an open channel blocking reaction is discussed.

4 Quinidine also caused a 5 mV hyperpolarizing shift of the steady-state inactivation curve and increased the half-time for recovery from inactivation. Quinidine did not affect the onset of inactivation measured at −30 mV.

5 Internal quinidine did not appear substantially to affect either the peak amplitude or kinetics of I_K(f).

6 A study of some structural analogues showed that hydroquinidine and quinacrine had effects similar to those of quinidine. The effect of quinacrine on the amplitude and kinetics of I_K(f) was also pH-dependent. Cinchonine, which bears a close structural resemblance to quinidine, was much less effective as a blocker of I_K(f).

Keywords: Fast transient K^+ current; melanotroph; quinidine; quinacrine

Introduction

Quinidine is a clinical anti-arrythmic drug which affects ionic currents in heart muscle and which has also been shown to be a potent blocker of several classes of K^+ channel in a variety of cell types (e.g., Fishman & Spector, 1981; Hermann & Gorman, 1984; Imaizumi & Giles, 1987; Glavinović & Trifaró, 1988). As part of the pharmacological characterization of the channels subserving the transient outward potassium current (I_K(f)) in rat melanotrophs (Kehl, 1989; 1990) the effects of quinidine and some of its structural analogues were investigated. The results described here show that quinidine blocks I_K(f) and also affects the rate of decay of the current.

Methods

Recordings were made from acutely dissociated melanotrophs. These were obtained following the enzymatic treatment and mechanical disruption of the neuro-intermediate lobe of the pituitary gland of the adult male rat (Wistar, 200–300 g), as described previously (Kehl, 1990). Cells were held in tissue culture medium (Dulbecco's modified Eagle's medium: Ham's F-12 = 1:1) at 35°C in an atmosphere of humidified air for 1–10 h before use.

Macroscopic (whole-cell) currents were recorded at room temperature (20–25°C) by conventional patch electrode techniques. Patch electrodes pulled from Corning No. 7052 glass (A-M Systems, WA, U.S.A.) had a resistance of 3–5 MΩ and were coated at the tip with Sylgard (Dow Corning) to reduce capacitive transients. Unless indicated otherwise the holding potential was −70 mV. Current and voltage signals from a LIST EPC-7 amplifier were stored on video cassette tape and analysed off-line with a Labmaster A/D computer interface (Scientific Solutions, OH) and Indec BASIC-Fastlab routines (Sunnyvale, CA). For the calculation of the current density the membrane capacitance was read from the front panel setting of the amplifier and the specific capacitance was assumed to be 1 $\mu F\,cm^{-2}$.

The control recording solution contained (mM): NaCl 120, KCl 3.5, $MgCl_2$ 1.0, $CaCl_2$ 2, N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) 10, glucose 5 and tetraethylammonium (TEA^+) chloride 20 to block the slowly-activating, slowly-inactivating K^+ current (I_K(s)). The Na^+ and Ca^{2+} currents (McBurney & Kehl, 1988) were blocked with $CdCl_2$ 0.3 mM and tetrodotoxin 1–2 μM, respectively. The patch pipette (internal) solution contained (mM): KCl 120, $MgCl_2$ 5, ethyleneglycol-*bis*-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) 10, HEPES 10 and Na_2ATP 1. The pH of the external and internal solutions was adjusted to 7.4 with NaOH and KOH, respectively.

Drugs were obtained from Sigma or Aldrich. Solutions containing quinidine sulphate or structural analogues of quinidine were prepared fresh from 10 mM stock solutions stored at 4°C. Experiments were performed under dimmed lights to prevent photoinactivation of the test drugs. Solution changes were carried out with a stop-flow system in which the test solution flowed into one end of the recording chamber while at the other end solution was drawn off at a rate sufficient to maintain a constant depth of fluid. Once complete replacement of the bath solution had been achieved the flow was stopped and recordings were made.

Curve fitting was done by non-linear regression routines in BASIC-Fastlab or SYSTAT (Evanston, IL). Values are expressed as mean ± the standard error of the mean (s.e.mean).

Results

Quinidine reduces the peak amplitude of $I_K(f)$

In control medium the threshold for activation of I_K(f) was between −40 and −20 mV and the peak amplitude of the current increased monotonically with membrane depolarization (Figure 1a, circles; see also Kehl, 1989). Bath application of quinidine had two simultaneous effects. The rate of decay of I_K(f) increased and there was a reduction of the peak amplitude of evoked currents. The effect of quinidine on the amplitude of I_K(f) will be described first.

The current/voltage relation of Figure 1a indicates that the block of I_K(f) was concentration-dependent but only weakly voltage-dependent, if at all. For example, in 50 μM quinidine

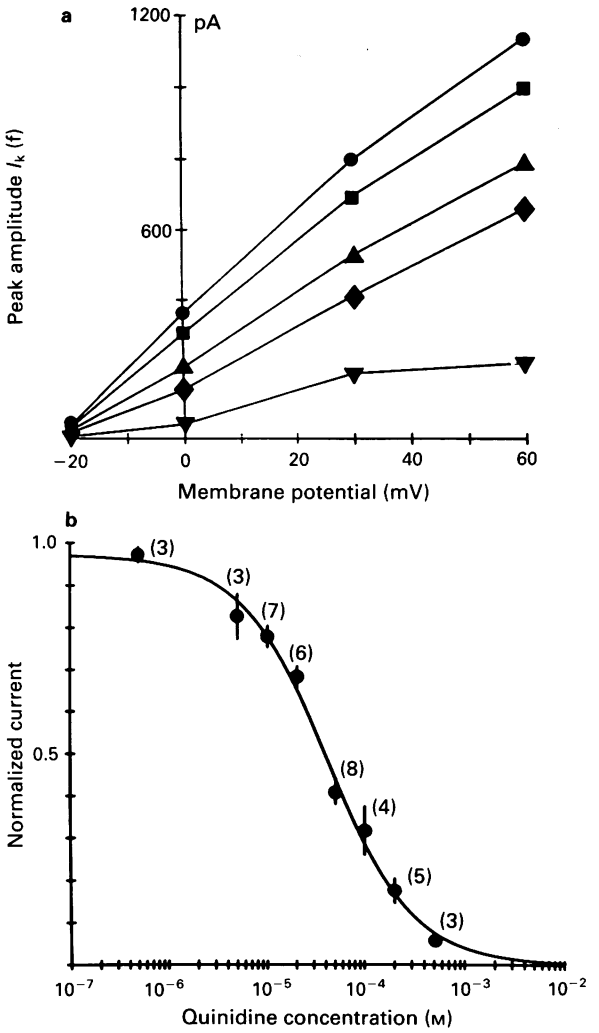

Figure 1 (a) The current/voltage relationship in 0 (●), 5 (■), 25 (▲), 50 (◆) and 250 (▼) μM quinidine. The peak amplitude of I_K(f) is plotted against the membrane potential during the test pulse. (b) The relationship between the peak amplitude of the I_K(f) and the concentration of external quinidine. The graphed points represent the mean with s.e.mean shown by vertical lines; the numbers in parentheses indicate the number of cells tested. The solid line represents the solution for the Hill equation in which K_d is 41 μM and n is 0.99.

(Figure 1a, diamonds) there was approximately a 50% reduction of the current at 0, 30 and 60 mV. The latency of onset for the effect of quinidine was short (<20 s with high concentrations) and a steady-state effect was obtained after a 1–2 min exposure to the drug. This inhibition of I_K(f) by quinidine could not be shown to be use-dependent, i.e., increasing the stimulus frequency to 1 Hz had no effect on the degree of block (not shown).

To quantify the actions of quinidine the amplitude of I_K(f) was measured from the difference current obtained by subtracting conditioned test currents from unconditioned test currents. Test pulses consisted of a step of the membrane potential from −70 mV to 0 mV for 300 ms. Conditioning pulses were applied to inactivate I_K(f) and reveal the leak current and any residual I_K(s). The conditioning pulse consisted of a 500 ms voltage step from −70 mV to −20 mV applied immediately before the test pulse. The cycle length was 4 s. After obtaining the controls the external solution was changed to one containing a known concentration of quinidine. Because the effect of quinidine treatment reversed slowly and was rarely completely reversible (see also Lee *et al.*, 1981; Hermann & Gorman, 1984) no attempt was made to obtain recovery responses before changing to a higher quinidine concentration. The decline of the current amplitude in quinidine did not appear to reflect cell deterioration since I_K(f) in quinidine-treated cells was stable for up to 1 h (the longest period tested) and I_K(f) could be recorded in cells which had been exposed to 50 μM quinidine for up to 5 h. Since quinidine did not alter the reversal potential of I_K(f) (data not shown) the inhibition of the current represents a conductance decrease.

The dose-response curve of Figure 1b was constructed by plotting the normalized amplitude ($I_{quinidine}/I_{control}$) of I_K(f) against the quinidine concentration. The data points were fitted by a least squares regression routine to the Hill equation:

$$I = (1 + (K_d/[Q])^{-n})^{-1}$$

where I is the normalized current, K_d is the apparent equilibrium dissociation constant and n is the order of the binding reaction. The K_d was 41 ± 5.6 μM (95% confidence limits 30.1–52.0 μM). The value for n was 0.985 ± 0.1 (95% C.L. 0.78–1.19) suggesting a first order reaction between quinidine and its binding site.

Quinidine increases the rate of decay of $I_K(f)$

Superimposed traces of control responses and those recorded at different quinidine concentrations showed that the decay of I_K(f) was accelerated while the rising phase of the current was only slightly affected by quinidine (e.g., Figure 4b). This increased rate of decay of I_K(f) in quinidine could arise from two mechanistically distinct (though not mutually exclusive) processes: (1) the blockade of open channels, subsequently referred to as open channel block; and (2) by an increase of the rate of channel inactivation. The possibility that quinidine was affecting inactivation was examined first.

Quinidine causes a hyperpolarizing shift of the steady-state inactivation curve for $I_K(f)$

By use of a two pulse protocol, steady-state inactivation was measured in 5 cells exposed to 100 μM quinidine. The data, when fitted to the Boltzmann equation by a non-linear least squares routine, gave a half-inactivation potential (V′) of −42 ± 0.6 mV and a slope factor (s) of 4.7 ± 0.5 mV (Figure 2a, squares). In control cells V′ and s were −37.5 ± 0.2 mV and 3.8 ± 0.1 mV, respectively (n = 6, Figure 2a, circles). The leftward shift of the inactivation curve in quinidine cannot account for the decline of the peak current shown in Figure 1a, since inactivation in quinidine, at least in 100 μM or lower concentrations, would be completely removed at the holding potential of −70 mV.

Onset of inactivation in quinidine

To determine if quinidine affected the kinetics of the onset of inactivation, a test pulse to 20 mV was preceded by a conditioning pulse to −30 mV lasting for 0–4 s. The rationale for this experimental approach was that at the potential of the conditioning pulse very few of the channels would open so that any change of kinetics would be due to a change of inactivation gating rather than open channel block (this assumes that the channels can inactivate directly from a closed (resting) state). The decline of the peak amplitude of the conditioned test currents both in control and 50 μM quinidine-treated cells was well-fitted by a single exponential with a time constant of 89 ± 16 ms (n = 3) and 94 ± 24 ms (n = 3), respectively. This indicated that inactivation at −30 mV was not affected by quinidine.

Recovery from inactivation in quinidine

Recovery from inactivation was investigated by use of a two pulse protocol in which 300 ms pulses to 10 mV were separated by a 5–2000 ms interval at −70 mV. A plot of the normalized peak current against the interval between pulses

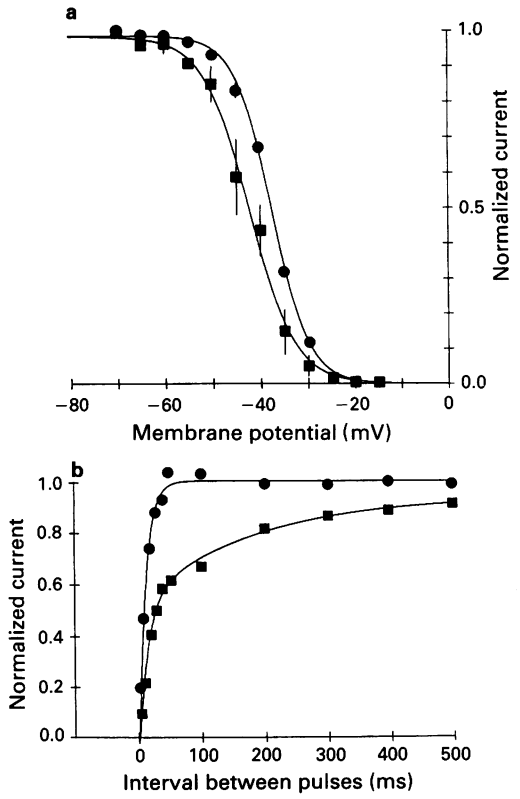

Figure 2 The effects of quinidine on steady-state inactivation and recovery from inactivation. (a) In a two-pulse protocol, a conditioning pulse to the potential indicated on the abcissa scale was applied for 500 ms immediately before the test pulse to 10 mV. Shown are the steady-state inactivation curves in control (●) and, in a separate group of cells, in 50 μM quinidine (■). The solid lines represent solutions to the Boltzmann equation in which V' and s were −37.5 mV and 3.8 mV in control cells ($n = 6$) and −42 mV and 4.7 mV in quinidine-treated cells ($n = 5$). (b) Quinidine slows recovery from inactivation. In control medium recovery from inactivation was well-fitted by a single exponential with a time constant of 12.5 ms (●). After changing to medium containing 50 μM quinidine, recovery was fitted by the sum of two exponentials with time constants of 14.5 and 180 ms (■). The slower component accounted for approximately 40% of the total current. A high room temperature (30°C) probably accounts for the faster than usual recovery from inactivation in the control cell.

showed in control cells that recovery from inactivation followed a single exponential (e.g. Figure 2b, circles) with a mean time constant of 25.7 ± 11.7 ms, $n = 4$ (half-recovery time of 17.9 ± 8.13 ms). In the same cells, after changing to 50 μM quinidine, recovery from inactivation was no longer well-fitted by a single exponential (Figure 2b, squares) and there was a wide variation in the half-recovery time (97.5 ± 68 ms).

Tail currents in quinidine

A common feature of many agents which block open channels is the alteration of tail current kinetics. This arises because the presence of the blocking particle in the channel pore hinders the 'closing' of the activation gate, i.e. the deactivation rate slows. The effect of 50 μM quinidine on the tail currents was examined in 43 mM external K^+ ($E_K \approx -30$ mV) since this allows a large inward tail current to be measured when the membrane potential is stepped to −70 mV from a potential where channels are open. For the traces illustrated in Figure 3 the membrane potential was stepped to 20 mV for 2–60 ms before returning to −70 mV. Note that the envelope of the peak tail currents accurately reflects the time course of $I_K(f)$ during the depolarizing pulse. This indicates that the tail current arises from channels which are open when the potential is suddenly changed to −70 mV and that the channels

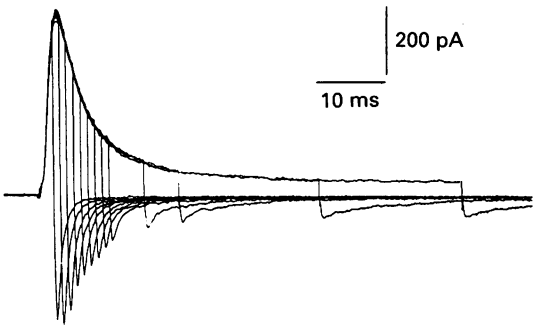

Figure 3 The $I_K(f)$ in 50 μM quinidine and 43 mM external K^+. Test pulses taking the potential from −70 mV to 20 mV lasted for 2–60 ms. Note the parallelism between the envelope of the peak inward tail current and the amplitude of the outward current prior to the end of the test pulse. This suggests that during the tail of current, quinidine-blocked channels do not become conductive prior to closure of the activation gate. The decay of the outward pulse current was well-fitted by a single exponential with a time constant of 4.6 ms. The slow tail current evident at the end of the 60 ms pulse is probably due to a residual $I_K(s)$.

which stopped conducting during the depolarizing pulse, either because of channel block or inactivation, do not re-open to contribute to the peak tail current. The deactivation time constant was not affected by quinidine (data not shown).

Since the results of these experiments indicated that quinidine either did not affect or slowed the onset of inactivation or recovery from inactivation, the simplest conclusion, particularly with the precedent for open channel block of Ca^{2+}-activated K^+ channels by quinidine (Glavinović & Trifaró, 1988), was that the increased rate of decay of $I_K(f)$ was due to an open channel blocking mechanism.

Open channel block by quinidine is concentration- and voltage-dependent

If quinidine blocks open channels then the rate constant for the decay ($1/\tau_{decay}$) of the macroscopic current will reflect the mean open time of the channel which is affected by two events, channel inactivation and open channel block for which the rate constants are, respectively, ($1/\tau_{inactivation}$) and ($1/\tau_{block}$). The relationship between these rate constants is,

$$1/\tau_{decay} = 1/\tau_{block} + 1/\tau_{inactivation}$$

The data shown in Figure 4 were generated from cells in which test pulses to 0, 30 and 60 mV were given in the presence of 5, 10, 50, 100 and 500 μM quinidine. The value of $1/\tau_{block}$ was calculated after fitting the decay of the membrane current to a single exponential to obtain $1/\tau_{decay}$ and using the time constant for inactivation in control medium to obtain $1/\tau_{inactivation}$. It should be emphasized here that although 50 μM quinidine was shown not to affect the onset of inactivation at −30 mV the assumption that the inactivation process is not affected at more depolarized potentials or with higher quinidine concentrations may introduce an unknown degree of error in the analysis. Additionally, the currents evoked in control cells at potentials of 30 mV or greater sometimes contained a second small exponential (which was not fitted) and there was a tendency for the time constant of the fitted exponential to increase slightly with increasing depolarization (e.g., Figure 7c, circles). It has not been possible to determine if this change of the kinetics of the control outward current with strong depolarizations reflects the gating of $I_K(f)$ channels or if there is a contribution from another channel type.

In the graph of Figure 4, which shows the relationship between the quinidine concentration and the blocking rate, the solid lines represent the best fit of a Michaelis-Menten type equation,

$$V = V_{max}/(1 + (K_m/[Q]),$$

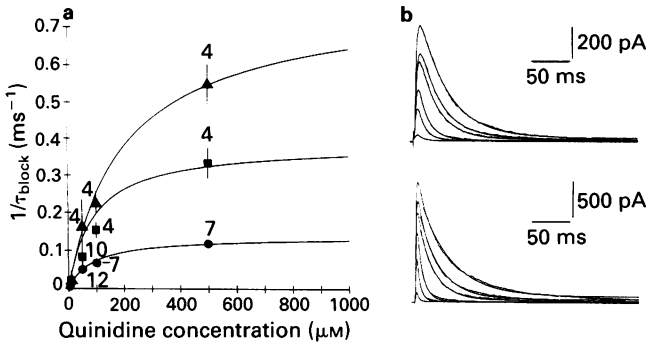

Figure 4 (a) The relationship between the concentration of quinidine and the estimated rate of blocking of open I_K(f) channels (see text for discussion). The solid lines represent the least squares fit to a Michaelis-Menten type equation. The values for V_{max} and K_m of the superimposed line are 0.14 ms^{-1} and 89 μM at 0 mV (●), 0.39 ms^{-1} and 48 μM at 30 mV (■) and 0.78 ms^{-1} and 106 μM at 60 mV (▲). The number adjacent to the symbol (mean with s.e.mean shown by vertical lines) indicates the number of cells tested. (b) Current traces, on which the graph is partially based, show, superimposed, responses in a cell depolarized to 0 mV (upper) and 30 mV (lower) in 0, 5, 10, 50, 100 and 500 μM quinidine. Exponentials superimposed on individual current traces had time constants of 37.8, 28.8, 24.7, 13.7, 10.1 and 7.9 ms (upper traces) and 39.8, 26.6, 20.4, 7.9, 4.9 and 3.1 ms (lower traces).

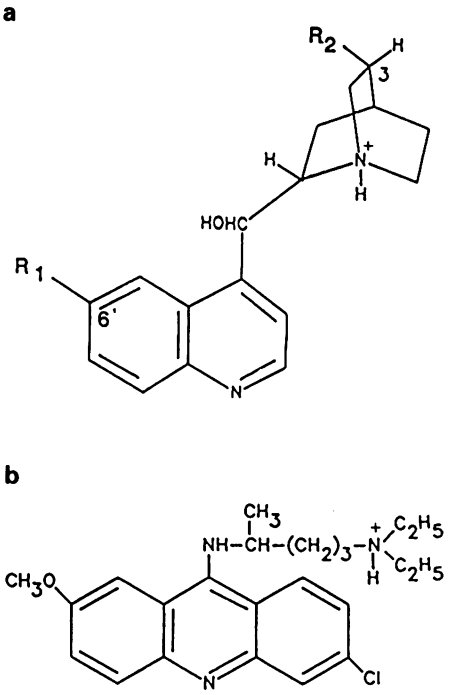

Figure 5 (a) The cinchona alkaloid structure on which quinidine, hydroquinidine and cinchonine are based. R_1 represents the group substituted on C-6' of the quinoline ring. R_2 represents the group substituted on C-3 of the quinuclide ring. For quinidine $R_1 = OCH_3$ and $R_2 = -CH{=}CH_2$; for cinchonine $R_1 = H$ and $R_2 = -CH{=}CH_2$; and for hydroquinidine $R_1 = -OCH_3$ and $R_2 = -CH_2{-}CH_3$. Each of the cinchona alkaloids is the 8S:9R isomer. (b) The structure of quinacrine.

where V_{max} is the maximal blocking rate and K_m is the concentration of quinidine ([Q]) at which the blocking rate ($1/\tau_{block}$) is half-maximal. The estimates of V_{max} and K_m were 0.14 ms^{-1} and 89 ± 16 μM at 0 mV, 0.39 ms^{-1} and 97.4 ± 16 μM at 30 mV, and 0.78 ms^{-1} and 212 ± 58 μM at 60 mV. These results indicate that the blocking rate increases with the concentration of quinidine and that depolarization accelerates the blocking rate.

Internal quinidine does not affect $I_K(f)$

With pK_a values of 8.3 and 4.2 for the nitrogen atoms on the quinuclide and quinoline groups of the quinidine molecule (Roche & Kier, 1981) (Figure 5a), then at pH 7.4 approximately 10% of the molecules exist in the uncharged hydrophobic form and might therefore diffuse through the membrane (Iwatsuki & Petersen, 1985). To assess the possibility that access to the site of action might be gained from the cytoplasmic face of the channel the effect of internal quinidine (at pH 7.4) was studied. With 100 μM quinidine inside the cell the decay of the current at 0 mV was not significantly affected (τ = 28.2 ± 3.9 ms, n = 13, vs. 32.5 ± 3.2 ms in 11 control cells). With 500 μM internal quinidine the decay of the current at 0 mV was still substantially slower (τ = 24.6 ± 1.2 ms, n = 4) than with 100 μM external quinidine (τ = 10.1 ± 0.6 ms, n = 7). The persistence of large outward currents at 0 mV with 500 μM internal quinidine (56.9 ± 13 μA cm^{-2}, n = 4 vs. 82.2 ± 29 μA cm^{-2} in 3 control cells and vs. 4.9 ± 2.2 μA cm^{-2} in 500 μM external quinidine, n = 3) also implied that there was little or no blocking effect of quinidine when it was applied internally.

Effect of structural analogues of quinidine

To gain some insight into the structural requirements for inhibition of I_K(f), the actions of some compounds related to quinidine (Figure 5) were tested.

Quinine, the stereoisomer of quinidine, reduced the amplitude of I_K(f) and accelerated the rate of the current decay. Quinine appeared to be as potent as quinidine but this was not quantified.

Cinchonine, which is identical to quinidine save for the substitution of hydrogen for the methoxy group on C-6' of the quinoline ring (Figure 5a) was, surprisingly, much less potent than quinidine. For example, 100 μM cinchonine reduced the peak amplitude of I_K(f) elicited at 0 mV by only 18 ± 3% (n = 6) and did not significantly affect the rate of the current decay at 0 mV (τ_{decay} = 37.8 ± 2.1 ms and 36.6 ± 1.2 ms in 0 and 100 μM cinchonine, respectively, n = 5). In three of these cells which were subsequently exposed to 100 μM quinidine τ_{decay} decreased to 12.4 ± 1.4 ms and the peak amplitude of I_K(f) decreased by 49 ± 3% (Figure 6a, traces). As with quinidine, with stronger membrane depolarization there was a reduction of τ_{decay}, an effect which may have contributed to the slightly greater inhibition of I_K(f) by cinchonine at positive membrane potentials in the current/voltage relation of Figure 6a.

Hydroquinidine, which differs from quinidine only at C-3 of the quinuclide ring where there is an ethyl group instead of an ethylene group (Figure 5a), affected I_K(f) in the same manner as quinidine and appeared to be of a similar potency. Half-maximal inhibition of I_K(f) at 0 mV occurred with 23 ± 7.8 μM hydroquinidine (95% C.L. 6.9–39.8 μM, n = 8) (traces of Figure 6b). The effect of hydroquinidine on τ_{decay} was identical to that of quinidine: the blocking rate was voltage-dependent and again the relationship between the blocking rate and the drug concentration was hyperbolic. The lines fitted to the data of the graph in Figure 6b represent solutions to the Michaelis-Menten type equation in which K_m at 0, 30 and 60 mV was 81.2 ± 19, 84.6 ± 15 and 81.2 ± 14.7 μM, respectively, and V_{max} was 0.1 ± 0.01, 0.28 ± 0.02 and 0.404 ± 0.03 ms^{-1}.

Quinacrine is an anti-malarial agent based upon the aminoacridine ring structure (Roche & Kier, 1981) (Figure 5b). The ring nitrogen has a pK_a of 8.2 whereas the pK_a of the tertiary amine of the side chain is 10.2. On the premise that the hydrophobicity of the ring might be an important determinant of binding, the actions of quinacrine were tested at pH 6.5 and pH 8.5 where the ratio of charged to neutral aromatic nitrogens would be approximately 50:1 and 1:2, respectively. The results of one of three such experiments are shown in Figure 7. At pH 6.5, 100 μM quinacrine had only a small effect on the amplitude and decay of I_K(f) evoked at 0 mV (Figure 7a, left traces). Switching to control solution with a pH of 8.5 (Figure

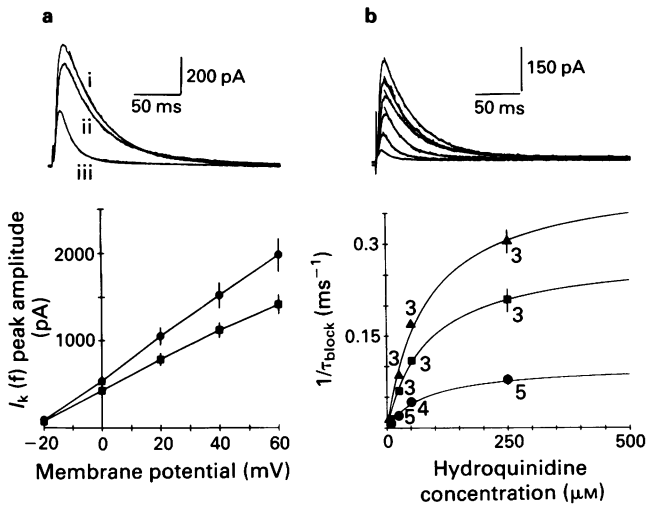

Figure 6 (a) Cinchonine is a poor blocker of I_K(f). The traces show the current evoked at 0 mV in control medium (i) and medium containing 100 μM cinchonine (ii) or 100 μM quinidine (iii). The graph below shows the current/voltage relationship derived from 5 cells in 0 (●) and 100 μM cinchonine (■). (b) Hydroquinidine reduces the peak amplitude of I_K(f) and increases its rate of decay. The traces illustrate the currents evoked at 0 mV in 0, 1, 5, 25, 50 and 250 μM hydroquinidine. The time constants for the superimposed exponentials are 37.9, 34.1, 34.5, 22, 15.4, and 11.7 ms. The lines in the graph were fitted as described in the text and represent solutions to a Michaelis-Menten type equation. The numbers adjacent to the symbols (mean with s.e.mean shown by vertical lines) indicate the number of cells tested.

7a, middle traces) had no discernible effect on either the amplitude or kinetics of I_K(f). At pH 8.5, however, 100 μM quinacrine reduced the amplitude of I_K(f) (205 ± 32 pA vs. 474 ± 6 pA in pH 8.5 control solution) and decreased τ_{decay} (24.2 ± 3 ms vs. 55 ± 6 ms). The effect of quinacrine reversed after returning to control medium at pH 6.5 (Figure 7a, right traces). As with quinidine there was no apparent voltage-dependence for the reduction of the amplitude of I_K(f) by quinacrine (Figure 7b, inverted triangle) but the rate of decay

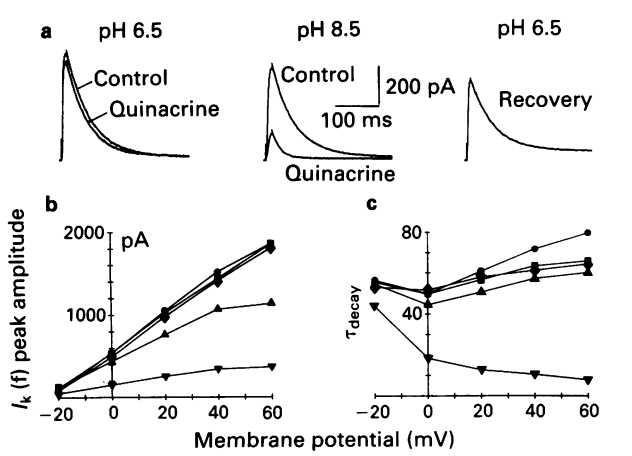

Figure 7 The block of I_K(f) by quinacrine is pH-dependent. (a) The traces show the currents evoked at 0 mV in control medium and 100 μM quinacrine. The time constants of the superimposed exponentials are 49.7 ms (control, pH 6.5), 44.4 ms (100 μM quinacrine, pH 6.5), 49.6 ms (control, pH 8.5), 18.4 ms (100 μM quinacrine, pH 8.5) and 51.5 ms (recovery, pH 6.5). The graphs show the current/voltage relationship (b) and the τ_{decay}/voltage relationship (c) in the control medium at pH 6.5 (●), in 100 μM quinacrine at pH 6.5 (◆), in control solution at pH 8.5 (■), in 100 μM quinacrine at pH 8.5 (▼), and recovery at pH 6.5 (▲). Note that at pH 6.5, quinacrine slightly reduces the amplitude of I_K(f) and slightly decreases τ_{decay}. At pH 8.5, however, there is a large reduction of the current amplitude and τ_{decay} shows a marked voltage-dependence.

of I_K(f) did increase with depolarization (Figure 7c, inverted trian[illegible]).

Discussion

It has been shown that I_K(f) in melanotrophs is blocked by quinidine. Quinidine also causes a small hyperpolarizing shift of the steady-state inactivation curve and slows the recovery of I_K(f) from inactivation. Quinidine exerts similar actions on the Na^+ current (Lee *et al.*, 1981) and the fast outward K^+ current (Imaizumi & Giles, 1987) in cardiac myocytes. Although having no apparent effect on activation or deactivation kinetics, the slowing of recovery from inactivation implies that the occupation by quinidine of its binding site can affect the operation of the inactivation gate.

In addition to reducing the peak amplitude of I_K(f), quinidine accelerated its rate of decay. It is not clear how these two effects are related. It is most unlikely however that the decline of the current amplitude is due solely to the increased rate of current decay. This suggestion is based on the output of computer simulations shown in Figure 8. To generate the traces of Figure 8d quinidine was constrained to bind only to open channels and so cause open channel block (B_{oc}). For the traces of Figure 8c it was assumed that the binding of quinidine to a site on closed (resting) channels would prevent channel opening and thereby reduce the amplitude of I_K(f) in a concentration-dependent manner. This represents the process referred to subsequently as tonic block (B_t). The traces of Figure 8b were derived from a model incorporating both tonic and open channel block. In modelling the current behaviour it was assumed that channel inactivation was coupled to channel opening:

$$\begin{array}{ccccc} & & I & & \\ & & \Downarrow\Uparrow & & \\ B_t & \rightleftharpoons & C & \rightleftharpoons & O \\ & & \Downarrow\Uparrow & & \\ & & B_{oc} & & \end{array}$$

The values for the rate constants for the channel gating reactions were derived from control currents at 0 mV and are given in the legend of Figure 8. The degree of tonic block was calculated from the Hill equation fitted to the data of Figure 1b. The rate constant for the open channel blocking reaction was derived from the data obtained at 0 mV in Figure 4 (circles).

A comparison of the outcome of the different blocking models with experimentally-derived traces (Figure 8a) shows that the model incorporating only an open channel blocking mechanism (Figure 8d) caused the expected increase in the rate of the current decay but produced, at most, only a 20% reduction of the peak amplitude of the current. Because open channel block alone does not reproduce the experimentally observed decline of the peak amplitude it is necessary to invoke an additional blocking mechanism, the tonic block. Indeed in the model incorporating both open channel block and tonic block the output of the simulation (Figure 8b) most closely resembled the experimental responses (Figure 8a). In Figure 8c, which shows the output of the model with tonic but with no open channel block, the amplitudes of the currents are comparable to those in Figure 8a and 8b (which is consistent with the relatively minor effects of open channel block on peak amplitudes in Figure 8d), but the decay of the current in quinidine is slower than that observed experimentally.

Although the data of Figure 8 show clearly that open channel block cannot in itself account for the effects of quinidine on the amplitude of I_K(f), the mechanistic basis for the tonic block remains unknown. Amongst the possibilities are a reduction of the probability of channel opening and/or a decrease of the single channel conductance.

The analysis of I_K(f) in quinidine indicated: (1) that at a given voltage the rate of decay of I_K(f) increased with the

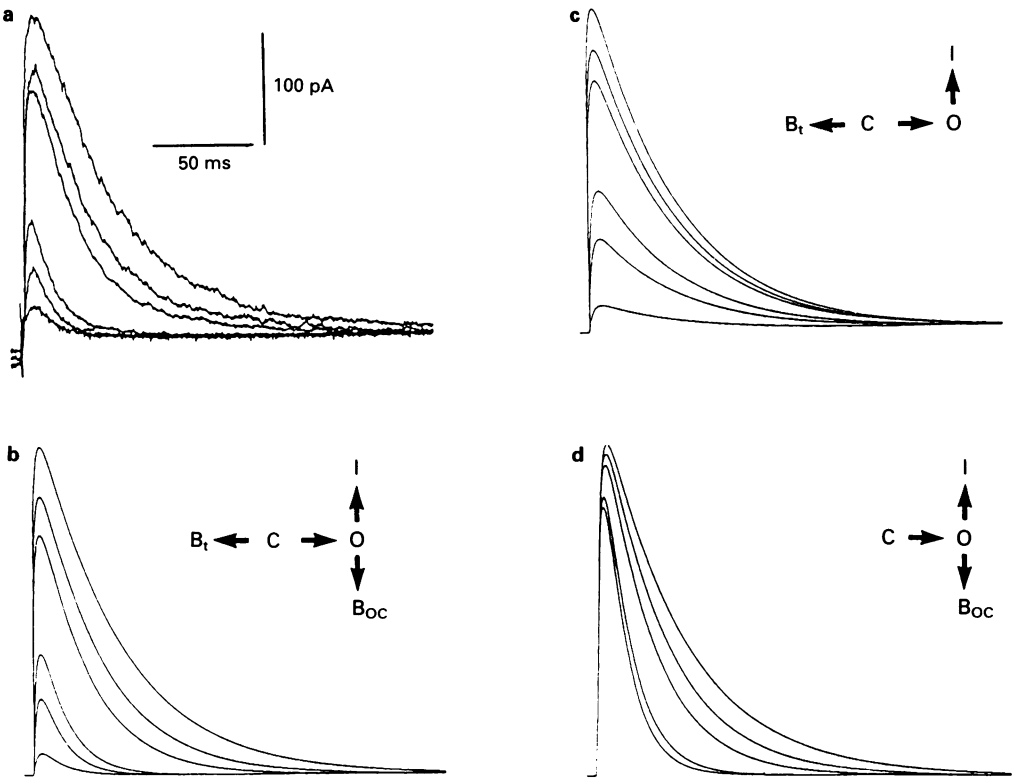

Figure 8 Superimposed traces of $I_K(f)$ obtained experimentally (a) or from a kinetic model in which, in addition to the normal course of channel activation and inactivation, there was: (b) tonic (B_t) and open channel (B_{oc}) block; (c) tonic channel block only; and, (d) open channel block only. The experimental and computer-generated data represent responses at 0 mV in 2.5, 5, 25, 50 and 250 μM quinidine. Although the amplitudes of the traces both in (b) and (c) are comparable to those of the experimentally-derived currents, the rate of decay of the current is better matched when open channel block is incorporated (b). The traces shown in (d) indicate that open channel block by itself has a relatively small effect on the amplitude of $I_K(f)$ despite the increased rate of decay of the current. The values for the forward rate constant for the channel blocking reaction (O–B_{oc}) were calculated from the curve fitted to the data obtained at 0 mV in Figure 4a; the rate constants for channel activation (C–O) and inactivation (O–*I*), estimated from control responses, were 0.5 and 0.033 ms^{-1}, respectively. Tonic channel block (C–B_t), estimated from the curve of Figure 1a, was mimicked by reducing the number of closed (resting) channels available for activation at the beginning of the test pulse. The time scale for the computed traces is the same as in (a); the output of the computer model was scaled so that the amplitude of the control response matched the peak of the control current in (a).

quinidine concentration; and (2) that for a given concentration of quinidine the rate of decay of $I_K(f)$ increased with membrane depolarization. There are a number of schemes which might account for the increase of the decay rate in quinidine. One possibility which cannot be ruled out is that quinidine increases the rate of channel inactivation. However, since the onset of inactivation at −30 mV was not affected by quinidine and since in control cells inactivation is only weakly voltage-dependent above 0 mV (Kehl, 1989) this seems unlikely.

I reported previously that 50 μM quinidine increased the rate of decay of the current at 50 mV but had little effect on the peak amplitude of the current (Kehl, 1989). There is no simple explanation for the failure to detect the tonic block in the previous study. One possibility is that in the previous experiments the quinidine (which was applied with a 'U-tube' tool) was not achieving the expected concentration near the cell, perhaps because of unstirred layer effects. Indeed in this study with 5 μM quinidine it was possible consistently to increase the rate of decay of the current at 50 mV while causing only a small decrease of the peak current.

If the quinidine binding site is situated within the lumen of the channel in such a way that access to the site is normally prevented by a physical barrier (such as the closed 'activation gate'), then once the channel is open quinidine would be able to diffuse through the aqueous phase to its binding site. Since at pH 7.4 most of the quinidine molecules would be positively charged and if the quinidine binding site is within the electric field of the membrane then the rate constants for the binding and unbinding reactions would be expected to be influenced by the membrane potential (Woodhull, 1973). An interaction between positively-charged quinidine and the electric field should be manifest as an inhibition of quinidine binding with membrane depolarization but in fact the opposite is observed: membrane depolarization apparently increases the blocking rate (Figure 4a).

The effects of quinidine on $I_K(f)$ are reminiscent of the actions of local anaesthetics on other types of voltage-gated currents. For example, in the squid axon the block of the Na^+ current by quinidine (Lee *et al.*, 1981) and quaternary lignocaine derivatives appears also to arise from tonic and phasic block possibly by 'different mechanisms [and receptors?]' (Cahalan & Almers, 1979). The basis for the tonic block of the Na^+ current by quinidine and local anaesthetics remains controversial. Phasic block, which for Na^+ currents is evident as a frequency-dependent decline of the current amplitude on successive pulses of depolarizing trains, has been attributed to a state-dependent binding reaction in which the drug binds to open and/or inactivated but not resting channels. It is very likely that part of the effect of quinidine reported here also requires channel opening, the principal differences being, first, that the effect of the drug is evident during each depolarizing pulse and, second, the unbinding reaction at −70 mV is rapid (relative to recovery from inactivation) so that a frequency-dependent decline of the current is not observed. Interestingly, another cardiac anti-arrhythmic agent, tedisamil, has recently been reported to speed the inactivation of the transient potassium current in ventricular myocytes (Dukes & Morad, 1989).

Internal quinidine had little or no effect on either the peak amplitude or the rate of decay of $I_K(f)$. This information may

provide a useful clue regarding the channel dimensions. For example, if quinidine acts by binding to a site within the lumen of the channel then the failure of internal quinidine to affect the rate of decay of I_K(f) implies that the dimensions of the inner mouth of the channel prevent the entry of the quinidine molecule into the pore. A depot site for quinidine in the membrane phase near the channel protein, such as has been proposed to account for the actions of some local anaesthetics (Hille, 1977), also seems unlikely given the ineffectiveness of internal quinidine. The simplest conclusion is that the quinidine binding site is on the external face of the membrane. The short latency of onset with external quinidine is consistent with the latter conclusion and, interestingly, it has been reported that external but not internal quinine blocks open Ca^{2+}-activated K^+ channels in molluscan neurones, chromaffin cells and red blood cells (Hermann & Gorman, 1984; Glavinović & Trifaró, 1988; Reichstein & Rothstein, 1981). This suggests that a quinidine-binding site may be a structural element common to transient and Ca^{2+}-activated K^+ channels.

The quinidine molecule consists of a hydrophobic portion, the aromatic quinoline ring, and a quinuclide ring bearing a nitrogen which would be protonated at pH 7.4. This arrangement in which a hydrophobic group is separated by a carbon chain from a charged nitrogen resembles the optimum configuration for open channel block of delayed rectifier channels in the squid giant axon by quaternary ammonium (QA) ion derivatives (Armstrong, 1971). In Armstrong's model it was proposed that a configuraton in which a hydrophobic benzene ring is separated by a 3 carbon chain from a quaternary nitrogen acts to stabilize the QA-blocking site complex. The influence of the pH on the actions of quinacrine is also consistent with the notion that the aromatic ring plays an important anchoring or stabilizing role at the site to which quinidine and its analogues bind.

The failure of cinchonine to mimic the actions of quinidine was somewhat surprising given the slight structural difference between the two molecules. These results suggest that the methoxy group on C-6′ of the quinoline ring is required for activity but it is not clear why this is so. This substitution at C-6′ would be expected to cause only a slight decrease of hydrophobicity.

The ethylene group on the quinuclide ring of quinidine and indeed the quinoline ring itself does not appear to be critical for the blocking reaction since hydroquinidine and quinacrine are also effective.

Quinidine blocks I_K(f) in melanotrophs but it is not a selective blocker for this channel. The slowly-activating, slowly-inactivating potassium current (I_K(s)) in melanotrophs is also potently blocked by quinidine (Kehl, unpublished observations). Nonetheless quinidine may be a useful tool for discriminating subclasses of fast transient K^+ currents based on the mechanism(s) of channel blockade. In this connection the transient outward K^+ current in cardiac myocytes is blocked by quinidine ($K_d \approx 7\,\mu M$) but there is no clear indication that this effect is accompanied by an increase of the rate of decay of the current (Imaizumi & Giles, 1987).

Supported by a Scholarship from the B.C. Health Care Research Foundation and operating grants from BCHCRF and the Medical Research Council of Canada. I am grateful to Dr P. Vaughan and Dr D. Mathers for comments on the manuscript.

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(Received March 8, 1991
Accepted March 19, 1991)