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Quinidine-induced open channel block of K^+ current in rat ventricle

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1 The effects of quinidine on calcium-independent outward K^+ currents in rat ventricular myocytes were studied using whole-cell patch clamp techniques.

2 Quinidine sulphate (6 μM) significantly prolonged repolarization of the ventricular action potential. This effect was larger during early repolarization (25% level) than at later times (90% level).

3 Quinidine reduced the amplitude of a transient outward current, and accelerated its rate of decay by approximately 4 fold at membrane potentials between 0 to +50 mV. Quinidine also reduced the amplitude of a slowly inactivating, tetraethylammonium-sensitive 'pedestal' component of the outward current.

4 The quinidine-induced block of the transient outward current was dependent on time and membrane potential. Maximal block occurred with depolarizations of about 100 ms duration, and longer depolarizations (up to 1.5 s) produced little additional block. The membrane potential dependence of quinidine-induced block was very similar to the membrane potential dependence of activation of the transient outward current. The membrane potential dependence of steady-state inactivation of the transient outward current was not significantly affected by quinidine.

5 These results show that quinidine blocks outward K^+ currents in rat ventricular cells. The time and potential dependence of this block suggests that quinidine blocks the transient outward K^+ current by acting primarily on the open state of these channels.

Keywords: Voltage-clamp; K^+ current; antiarrhythmic drugs; repolarization

Introduction

Quinidine is an antiarrhythmic drug which has both class I and class III actions in mammalian atrium and ventricle (Spinelli & Hoffman, 1989; cf. Nattel, 1991; Rosen *et al.*, 1991). Although its ability to block sodium channels (class I antiarrhythmic actions) has been described in some detail as a use-dependent inhibition (Hille, 1977; Hondeghem & Katzung, 1977; 1984; Clarkson & Hondeghem, 1984), the action potential broadening, or class III actions of quinidine are less well understood (Singh & Nadamane, 1985; Hondeghem & Snyders, 1990; Colatsky *et al.*, 1990; Lynch *et al.*, 1992). Part of the reason for this is that in mammalian heart there is a relatively large number of quite diverse potassium (K^+) currents (Colatsky *et al.*, 1990; Baumgarten & Fozzard, 1991; Gintant *et al.*, 1991). Inhibitory actions of quinidine on a number of these K^+ currents have been described, including effects on the inward rectifier, I_{K1}, in Purkinje fibre and ventricle (Roden & Hoffman, 1985; Salata & Wasserstrom, 1988; Balser *et al.*, 1991b), the time- and voltage-dependent delayed rectifier potassium currents in guinea-pig ventricle (Hiraoka *et al.*, 1986; Roden *et al.*, 1988; Balser *et al.*, 1991a; Wettwer *et al.*, 1992) and rabbit sinoatrial and atrioventricular nodes (Furukawa *et al.*, 1989), the calcium-independent transient outward K^+ current in rabbit atrium (Imaizumi & Giles, 1987), canine cardiac Purkinje cells (Nakayama & Fozzard, 1987) and rat ventricle (Slawsky & Castle, 1994) and the ATP-sensitive K^+ current (Undrovinas *et al.*, 1990).

Previously Imaizumi & Giles (1987) have shown that quinidine is a potent, although somewhat nonselective, blocker of a calcium-independent transient outward current in rabbit atrium. Nakayama & Fozzard (1987) obtained somewhat similar data from a canine Purkinje fibre preparation. A consistent and quite striking feature of both of these sets of results was that when this transient outward K^+ current was inhibited by quinidine, the times course of decay was accelerated significantly, suggesting that quinidine may act as an open channel blocker (cf., Armstrong, 1971), as had been described previously for the actions of quinidine in neurones (Hermann & Gorman, 1984; Oyama *et al.*, 1992) and pituitary melanotrophs (Kehl, 1991). Recently, Snyders *et al.* (1992) have shown that quinidine can act as an effective open channel blocker of one of the delayed rectifier K^+ currents (HK2: hKv1.5) cloned from human ventricle when it is expressed in a mouse L cell line.

The main goal of this study was to determine the mechanism by which quinidine can block the outward potassium currents which repolarize mammalian ventricular muscle. Rat ventricular muscle was chosen since recent very detailed data describing the types, relative magnitudes, and kinetics of the potassium currents in this tissue were available (Apkon & Nerbonne, 1991). Our results show that in single myocytes from this mammalian ventricular tissue, quinidine is a potent blocker of both a transient outward potassium current and a slowly inactivating or 'pedestal type' of potassium current which is somewhat similar to hKv1.5 from human ventricle. Evidence is presented describing the mechanism of this open channel block. These results are compared to and contrasted with previously published actions of quinidine on K^+ channels in native membranes of heart cells, and cloned heart K^+ channels expressed in reconstitution systems (Snyders *et al.*, 1992; Yatani *et al.*, 1993).

Methods

Solutions

Standard HEPES-buffered Tyrode solution contained (mM): NaCl 140, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 10 and

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glucose 5.5. 'Low calcium' solution was prepared by omitting $CaCl_2$ from standard Tyrode solution. The 'enzymatic' solution used in cell dissociation contained (mM): NaCl 130, KCl 5, $MgCl_2$ 1, HEPES 10, glucose 5.5, taurine 10, collagenase (Type I; Sigma Chemical Co., St. Louis, MO, U.S.A.) 0.8 mg ml^{-1} and protease (type XIV; Sigma Chem. Co.) 60 $\mu g\ ml^{-1}$.

The 'washout' solution was a modification of standard Tyrode solution, in which $CaCl_2$ was reduced to 100 μM and 1 mg ml^{-1} bovine serum albumin (type V; Sigma Chem. Co.) was added. All solutions were gassed with 100% O_2 and pH was adjusted to 7.4 by addition of appropriate amounts of 1.0 M NaOH.

The control 'external' solution used in action potential recordings was the same as standard HEPES-buffered Tyrode. For voltage-clamp experiments, 3 mM $CoCl_2$ was added to block the voltage-dependent calcium current. 4-aminopyridine (4-AP) and tetraethylammonium chloride (TEA) were added to the external solution where indicated. In some experiments, tetrodotoxin (TTX; Sigma Chem. Co.) was added at a concentration of 10 μM. Quinidine sulphate (Sigma Chem. Co.) was added to the external solution to give a final concentration of 6 μM.

The pipette solution contained (mM): K-aspartate 70, KCl 70, $MgSO_4$ 1, KH_2PO_4 0.45, HEPES 10, EGTA 10 and Na_2-ATP 5. The pH of the solution was adjusted to 7.2 with appropriate amounts of 1 M KOH.

Cell dissociation procedure

The dissociation method was similar to that described by Clark *et al.* (1993). Rats weighing 250–350 g were killed by cervical dislocation. Their hearts were immediately removed and mounted on a modified Langendorff perfusion system for retrograde perfusion of the coronary circulation. Temperature was maintained at 36 ± 1°C throughout all perfusion steps. An initial 5 min period of perfusion with standard HEPES-buffered Tyrode solution was used to remove as much blood as possible from the heart. Three further steps of perfusion were as follows: 7 min with 'low Ca' solution, 15 min with 'enzymatic' solution and 5 min with 'washout' solution. The heart was then removed from the perfusion system, and the atria and ventricles were separated. Small pieces of the epicardium from the apex, and endocardium from the base of the left ventricle were placed separately in small flasks containing 'washout' solution, where the pieces were gently stirred until the fragments were dissociated into single cells.

Electrophysiological and data-recording procedures

A few drops of cell suspension were placed in a superfusion chamber (vol. 200 μl) mounted on the modified stage of an inverted microscope (IMT; Olympus, Toyko, Japan). The myocytes were left for several minutes to allow them to settle onto the bottom of the chamber. Thereafter, the cells were superfused (about 0.5 ml min^{-1}) with control 'external' solution. A multi-way valve was used to select either control or quinidine-containing solutions. Cells were exposed to quinidine-containing solutions for 5–10 min before recordings were taken. All experiments were carried out at room temperature (21–23°C).

Electrophysiological recordings were made from isolated myocytes using whole-cell patch clamp methods (Hamill *et al.*, 1981). Patch electrodes were made from borosilicate glass (World Precision Inst., Sarasota, FL., U.S.A.), and had tip resistances of 1–2 MΩ-when filled with pipette solution. Voltage and current-clamp experiments were carried out with a conventional patch-clamp amplifier (PC-501; Warner Instruments, Hamden, Conn., U.S.A.). Pipette series resistance in the whole-cell mode was 5–8 MΩ; 60–80% of this resistance was compensated electronically. Cell capacitance was usually not compensated. Data acquisition and generation of current and voltage-clamp command protocols was carried out with a combined A/D and D/A convertor board (Techmar), which was controlled by 'pCLAMP' software (Axon Instruments, Foster City, CA, U.S.A.). Data were stored on hard disk in a microcomputer (IBM) for later analysis. Current records were fit to exponential functions by a least-squares method programme ('Minsq', Micromath). Sets of data points were fitted to exponential and Boltzmann functions using a commercial graph plotting programme ('Sigmaplot', Jandel Scientific, Corta Madera, CA, U.S.A.). Average values are shown as mean ± s.e. Differences between control and quinidine-treated cells were evaluated statistically by Student's paired or unpaired t test, where appropriate; differences were considered to be significant for $P<0.05$.

Results

Effect of quinidine on rat ventricular action potentials

The average resting membrane potential of rat isolated ventricular cells, bathed in HEPES-buffered Tyrode solution containing 5 mM KCl, was −79 ± 2 mV (mean ± s.e., $n=6$). Action potentials in the isolated cells had both a rapid upstroke and initial repolarization followed by a second, slower phase of repolarization (Figure 1a). The average amplitude of the action potentials was 113 ± 3 mV, and the duration, measured at the 25% and 90% repolarization levels, was 7.0 ± 0.5 ms and 49 ± 8 ms, respectively when stimulated at 0.1 Hz. The characteristics of these action potentials from isolated myocytes were similar to those recorded from intact ventricular muscle.

Application of quinidine sulphate (6 μM) did not affect the resting potential of isolated myocytes (−78 ± 2 mV; $n=6$). The average amplitude of the action potentials was slightly but significantly ($P<0.05$) reduced (107 ± 4 mV; $n=6$). Examples of the effects of quinidine on the action potential of two different rat ventricular myocytes are shown in Figure 1a. The action potential in the presence of quinidine had a much slower rate of initial repolarization, which produced a more distinct early plateau region than in the controls. The late phase of repolarization was also prolonged by quinidine, although the effect on this phase of the action potential was less pronounced than on initial repolarization. In the presence of quinidine, action potential duration at the 25% repolarization level was 21 ± 3 ms, 3 fold larger than controls, while the duration at the 90% level was 73 ± 9 ms, an approximately 1.5 fold increase over control values. Both values were significantly ($P<0.05$) increased compared to controls.

Effects of quinidine on outward currents in rat ventricular myocytes

The broadening of the action potential in rat ventricular myocytes by quinidine suggested that part of its action may have resulted from inhibition of one or more of the components of outward current in these cells. It has been demonstrated previously (Apkon & Nerbonne, 1991) that outward currents in rat ventricular myocytes consist of at least two different time and voltage-dependent components with different pharmacological sensitivities to K^+-channel blockers, a rapidly activating and inactivating transient component which is blocked by 4-AP ('transient outward current') and a slowly inactivating 'pedestal' component which is sensitive to TEA. It was therefore of interest to compare the effects of 4-AP and TEA on quinidine-induced changes in action potential repolarization. Results from one experiment are shown in Figure 1b and c. Exposure of the cell to 4-AP (5 mM) produced a marked prolongation of the action potential (Figure 1b); addition of quinidine (6 μM) to the 4-AP-containing solution produced little additional effect. In con-

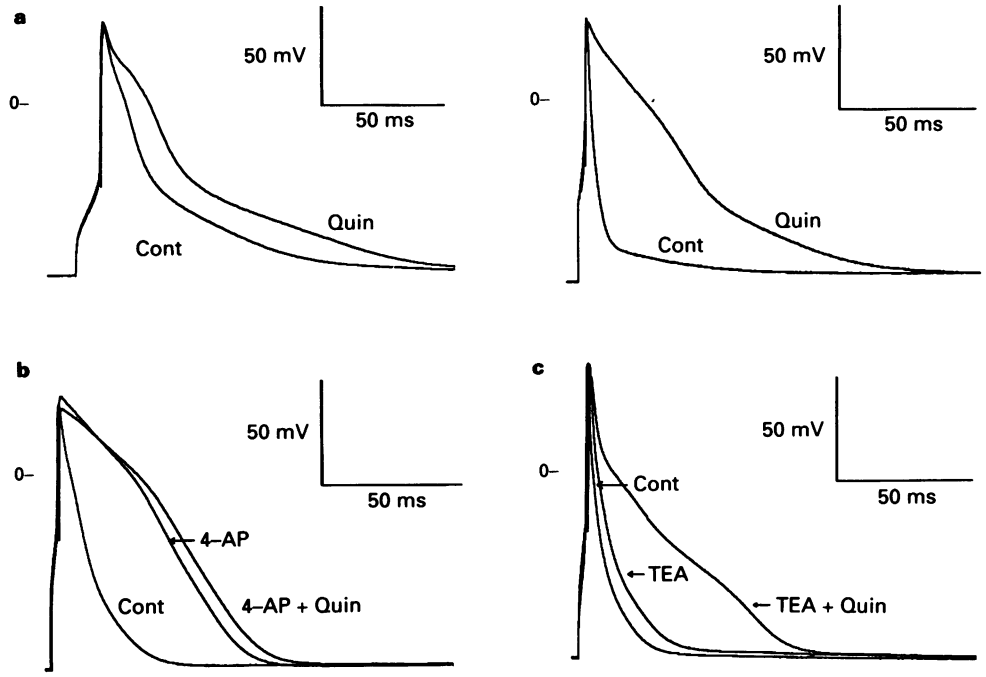

Figure 1 (a) Two examples of the effects of quinidine on the action potentials of different isolated ventricular cells of the rat. Action potentials were produced by 5 ms, 1 nA current pulses applied through the recording electrode at 0.1 Hz. Exposure to solution containing 6 μM quinidine sulphate (Quin) for 8 min resulted in a broadening of the action potential waveform. (b) Effect of 4-aminopyridine (4-AP) on the changes in action potential waveform induced by quinidine. An action potential was first recorded in control 'external' solution (Cont). External solution containing 5 mM 4-AP (4-AP) was then added, followed by a solution containing 4-AP and 6 μM quinidine sulphate (4-AP + Quin). (c) Effect of tetraethylammonium chloride (TEA) on the changes in action potential waveform induced by quinidine. Same cell as in (b). Action potentials were recorded in control solution, then 10 mM TEA, and finally in 10 mM TEA and 6 μM quinidine sulphate. The stimulation rate was 1 Hz in (b) and (c).

trast, exposure of the same cell to TEA (10 mM) alone produced a relatively small broadening of the action potential (Figure 1c), but addition of quinidine to the TEA solution resulted in a large additional prolongation.

Voltage-clamp experiments were carried out to assess the effects of quinidine on each of the outward currents of rat ventricular myocytes. An example of the quinidine-induced inhibition of the transient outward current in a rat ventricular myocyte is shown in Figure 2a. This experiment was done in the presence of 10 mM external TEA which blocked most of the slowly inactivating component of outward current. Quinidine (6 μM) reduced the amplitude of the transient outward current by about 15–20%. Figure 2b shows the effect of quinidine on the current-voltage relation for the peak transient outward current for membrane potentials in the range −30 to +50 mV, averaged from 8 cells taken from the epicardium of the apex of the left ventricle. Previous studies (Clark *et al.*, 1993) have shown that the transient outward current is largest in cells taken from this region of the rat heart. Note that quinidine reduced the amplitude of the transient outward current at all potentials over this range.

Quinidine also reduced the magnitude of the slowly inactivating outward current in rat ventricular myocytes, as shown in Figure 3a. This experiment was done on a myocyte taken from the endocardium of the base of the left ventricle; myocytes from this region of the rat heart have very little transient outward current (Clark *et al.*, 1993). Note that quinidine reduced the magnitude of the slow outward current in this cell by about 50%. Figure 3b shows that quinidine (6 μM) reduced the amplitude of the slowly inactivating outward current by about 50% at membrane potentials positive to −10 mV. These data were averaged from 6 endocardial/base cells in which the transient outward current was negligible.

Imaizumi & Giles (1987) noted that quinidine increased the rate of decay of a calcium-independent transient outward current in rabbit atrium. Quinidine produced a similar increase in the rate of decay of the transient outward current in rat ventricle, as described recently by Slawsky & Castle (1994). This can be seen in Figure 4a, where transient outward currents produced by a voltage-clamp step from −70 to +30 mV have been superimposed to compare the time course of decay of the current in control and in the presence of quinidine. The time course of decay of the current was fitted to an exponential function; the time constant was 41.3 ms in the control and was 18.6 ms after application of quinidine. Figure 4b shows dependence of the decay time constant on membrane potential in control conditions and after addition of 6 μM quinidine, averaged from 4 cells. In controls the time constant was 35–45 ms and was not significantly dependent on membrane potential in the range from −10 to +50 mV. The magnitude of the time constant was significantly reduced in the presence of 6 μM quinidine at membrane potentials positive to −10 mV, and decreased in magnitude by about 4 fold for membrane potentials over the range 0 and +50 mV.

Use-dependent block of the transient outward current by quinidine

The effectiveness of quinidine in blocking voltage-dependent sodium channels is increased if the channels are repetitively activated by trains of brief membrane depolarizations (Hondeghem & Katzung, 1984). It was of interest to see if quinidine produced a similar 'use-dependent' block of the transient outward current in rat ventricular cells. Figure 5a illustrates an example of results from an experiment designed to test this possibility. The voltage-clamp protocol consisted of a train of 100 ms test steps to +50 mV, from a holding potential of −70 mV, applied at a frequency of 2 Hz. In the absence of quinidine, the peak current produced by each pulse remained constant during a train of 10 pulses. Following application of quinidine, the amplitude of current pro-

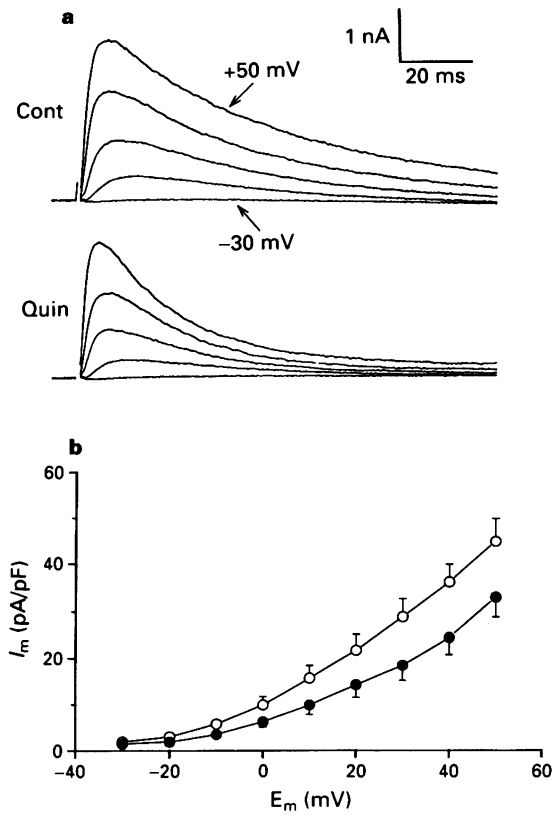

Figure 2 Effect of quinidine on transient outward currents in rat ventricle. (a) Example of transient outward currents, produced by voltage-clamp steps to −30, −10, +30 and +50 mV from a holding potential of −70 mV, in control conditions (Cont), and in the presence of 6 μM quinidine (Quin). The steps were applied at a frequency of 0.03 Hz. Note that quinidine reduced the amplitude of the current at all test potentials, and also increased the rate of decay of the current. The solutions in this experiment contained 10 mM tetraethylammonium chloride. The myocyte was taken from the epicardium of the apex of the left ventricle. (b) Averaged peak current-voltage relation for transient outward current, in control (○) and 6 μM quinidine (●). Peak current was normalized to cell capacitance for each cell. Mean ± s.e., $n = 8$.

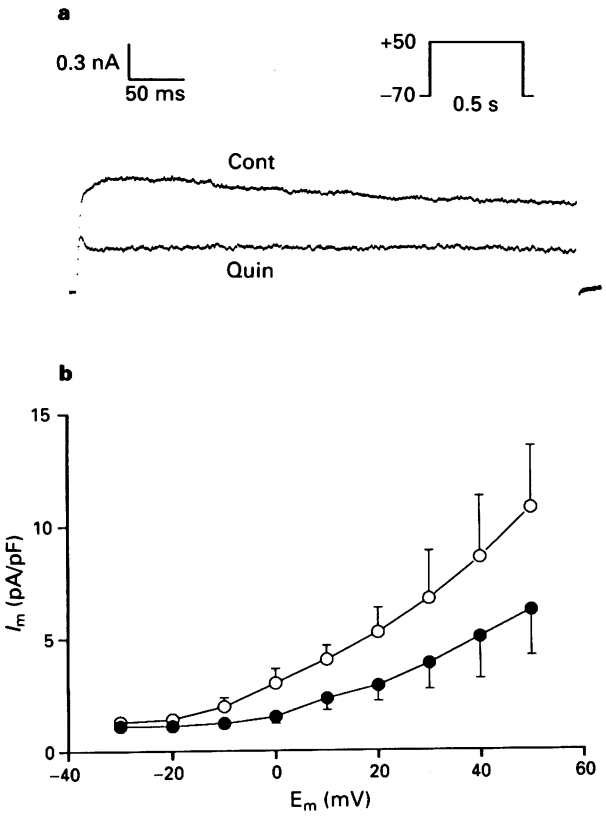

Figure 3 (a) Effect of quinidine on the slowly inactivating outward current in a voltage-clamped myocyte from the endocardium of the base of the rat left ventricle. The transient outward current was negligible in this cell. Quinidine (Quin) (6 μM) produced about 50% reduction in the current magnitude throughout the entire voltage-clamp step. (b) Averaged peak current-voltage relation for slowly inactivating outward current, in control (○) and 6 μM quinidine (●). Mean ± s.e., $n = 6$.

duced by the first pulse of the train was reduced by about 15%, and in contrast to the controls, the current amplitude declined with successive pulses in the train and reached a 'steady state' level of about 60% of the control amplitude after 8–10 pulses. Figure 5b shows averaged data from 8 cells, for pulse trains at frequencies of 0.5 and 2 Hz. The degree of inhibition of outward current amplitude per pulse was larger for the 2 Hz than the 0.5 Hz train, and the degree of 'steady state' block at 2 Hz was about twice that at 0.5 Hz. These use and frequency-dependent effects suggested that block of transient outward current by quinidine is related to states of the channels which are produced by membrane depolarization, i.e., open and inactivated states. The experiments described in the remainder of the paper were designed to elucidate the kinetic basis of the use-dependent quinidine block.

Time and membrane potential dependence of quinidine block of transient outward current

The data in Figure 5 show that in the presence of quinidine, even brief (e.g. 100 ms) depolarizations were sufficient to produce a substantial block of the transient outward current. Figure 6 shows the time-course of development of quinidine-induced block of transient outward current during membrane depolarization. This voltage-clamp protocol consisted of a pair of depolarizing steps to +50 mV from a holding potential of −70 mV. The first was a 'conditioning' step the duration of which was varied between 2 ms and 1.5 s, and the second was a 100 ms 'test' step. The conditioning and test steps were separated by a gap of 0.2 s at the holding potential; this allowed sufficient time for the channels which were not blocked by quinidine to recover from inactivation, but the gap was too short for quinidine-blocked channels to recover (see below). Hence comparison of the test current amplitudes in the presence and absence of the conditioning step gave a measure of the quinidine-induced block. In the absence of quinidine, this protocol produced only a small (<5%) depression of the test current amplitude as the conditioning step duration was varied from 2 ms to 1.5 s. However, in the presence of 6 μM quinidine, conditioning pulses as short as 5 ms produced a measurable decrease in test current amplitude. Conditioning pulses of longer duration resulted in greater depression of the test current, but with a 50 ms conditioning pulse a maximal depression of about 23% was reached and pulses as long as 1.5 s produced no significant additional block.

Figure 7a illustrates results from an experiment which compared the voltage dependence of activation, and quinidine-induced block, of transient outward current. A paired voltage-clamp protocol was used to measure the voltage-dependence of activation of the transient outward current. A 10 ms step to potentials between −30 and +70 mV was used to activate, without significantly inactivating, the transient outward current. The cell was immediately repolarized to −40 mV, and the amplitude of the tail current following repolarization was taken as a measure of transient outward current activation. Figure 7a

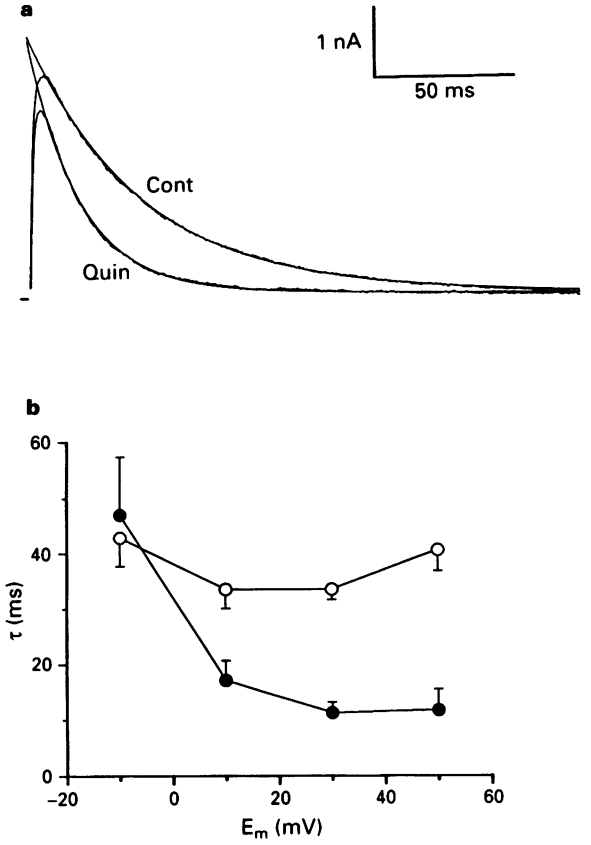

Figure 4 Effect of quinidine on the time course of decay of the transient outward current. (a) Superimposed current records in control and 6 μM quinidine, for voltage-clamp steps to +30 mV, from a holding potential of −70 mV. Single exponentials were fitted to the decay phase of the currents; the time constant for control was 41.3 ms, and in quinidine it was 18.6 ms. (b) Time constant of decay of the transient outward current vs. membrane potential, before (○) and after (●) 6 μM quinidine. Mean (± s.e.) values from 3 different control and 4 different quinidine-treated ventricular cells.

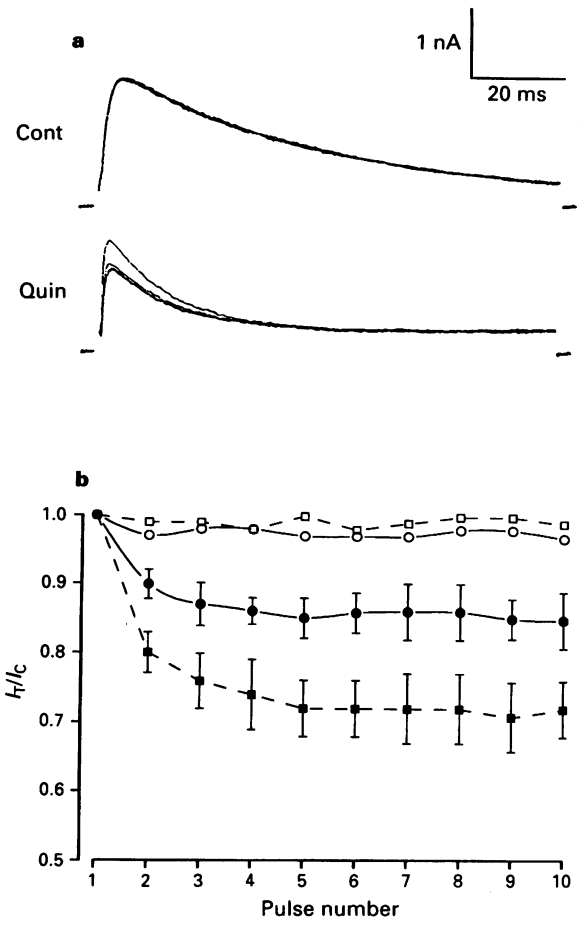

Figure 5 Use- and frequency-dependent effects of quinidine on transient outward current. (a) Effect of quinidine on the amplitude of transient outward current produced by a train of 10 voltage-clamp steps (100 ms; −70 to +50 mV), applied at a rate of 2 Hz. The upper records are 10 superimposed currents recorded in control conditions. Currents produced by the same train of steps after application of 6 μM quinidine are shown below. Transient outward current amplitude decreased with successive steps in the train. (b) Averaged (mean ± s.e.) data from 8 cells, for pulse train frequencies of 0.5 (○, ●) and 2 Hz (□, ■). The current amplitudes for each pulse in the train have been normalized to the first pulse (I_T/I_C). Note that there was no significant change in current amplitude in control (○, □) conditions, but in 6 μM quinidine, (●, ■) the current decreased with successive pulses and reached a 'steady-state' level by the end of the 10 pulse train, and that the block was greater for the 2 Hz train than the 0.5 Hz train.

shows the membrane potential-dependence of the tail current amplitude, normalized to its largest magnitude, at +70 mV (●). The potential dependence of activation was fitted to a Boltzmann function (see Figure 7a), with half-activation at −2 ± 0.5 mV, and a slope at half-activation of 12.7 ± 0.5 mV. The membrane potential-dependence of quinidine-induced block of the transient outward current was measured with the paired voltage-clamp protocol shown schematically in Figure 7a. A 'conditioning' step of 100 ms duration, at potentials between −60 and +80 mV, was followed 300 ms later by a 100 ms 'test' step to +50 mV. In the absence of quinidine, this protocol produced less than 5% depression of transient outward current at the most positive conditioning potentials. In the presence of 6 μM quinidine, conditioning depolarizations positive to about −40 mV produced measurable block of the transient outward current, and the amount of block increased with increased depolarization. Figure 7a shows that although activation of transient outward current and quinidine-induced block had similar membrane potential-dependence, activation was maximal for depolarizations to +40 mV, while the quinidine-induced block continued to increase over the range +40 to +80 mV. This effect may result from voltage-dependence of quinidine block of open transient outward current channels (see Discussion).

The time course of block of transient outward current by quinidine shown in Figure 6 approximately paralleled the inactivation time course of transient outward current (e.g. Figures 2 and 4), suggesting that the block occurred primarily during the time when the transient outward current channels were open; longer depolarizations which completely inactivated transient outward current produced no additional block (Figure 6). Additional data which suggest that quinidine does not significantly block inactivated channels are shown in Figure 7b, where the membrane potential-dependence of 'steady state' inactivation of the transient outward current in the presence and absence of quinidine are compared. 'Conditioning' steps of 1 s duration were applied over the potential range from −100 to −10 mV, followed immediately by a 'test' step to +50 mV. In control conditions, transient outward current was inactivated by conditioning potentials in the range from −60 to −10 mV, and the amplitude of transient outward current evoked by the test step was best-fitted by a Boltzmann function with half-inactivation at −32 ± 1 mV and a slope factor at half-inactivation of 3.9 ± 0.1 mV (see Figure 7b). In the presence of quinidine, there was no significant change in the potential dependence of inactivation; half-inactivation occurred at

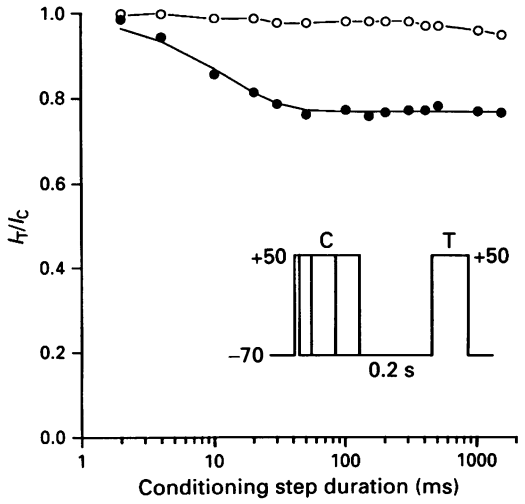

Figure 6 Time course of development of quinidine-induced block of transient outward current during depolarization. The voltage-clamp protocol is shown in the inset. A 'conditioning' step (+50 mV) of variable duration (2 ms to 1.5 s) was followed after a 200 ms interval at −70 mV by a 'test' step (+50 mV). Peak transient outward current in the presence of the conditioning step was normalized to the amplitude of transient outward current in the absence of the conditioning step. The plot shows the normalized amplitude of test current compared to control current (I_T/I_C), as a function of the conditioning pulse duration. Control (○), and 6 μM quinidine (●). Note that conditioning step duration is plotted on a logarithmic scale. The solid line fitted to the quinidine data is a single exponential function with a time constant of 12.1 ms.

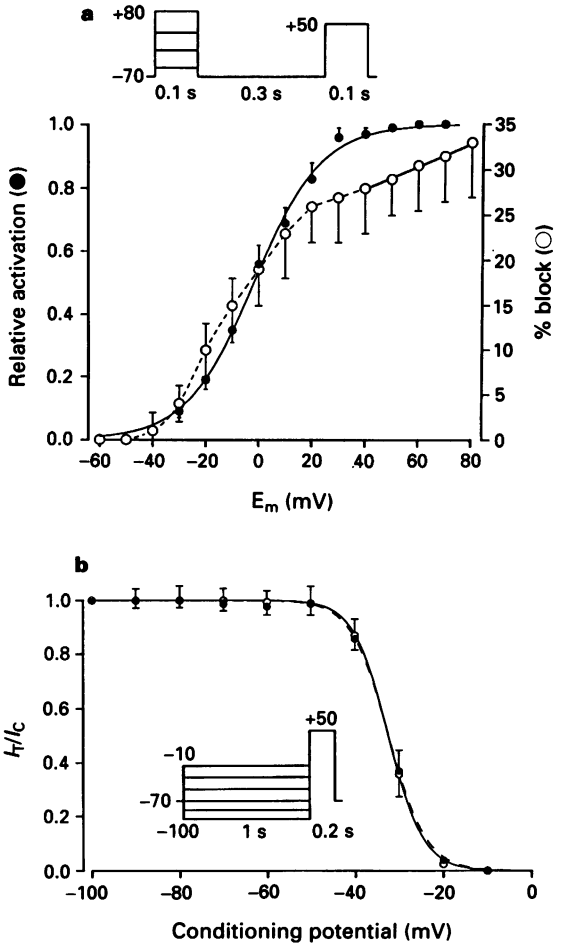

Figure 7 Voltage-dependence of quinidine-induced block of transient outward current. (a) Comparison of the membrane potential-dependence of activation of transient outward current (●), and quinidine-induced block (○). Activation of transient outward current was measured with a two-step voltage-clamp protocol (see text). The solid line is a Boltzmann function of the form

$$1/(1 + \exp[V_{1/2} - V_m)/S_{1/2}])$$

where V_m is membrane potential during the first voltage-clamp step. $V_{1/2}$ is the potential for half-activation, and $S_{1/2}$ is a slope factor at the half-activation potential. Data were pooled from 6 cells. Quinidine-induced block of transient outward current was measured using the voltage-clamp protocol shown; % block (○) was determined by comparing the 'test' (second step) current amplitude in the absence and presence of the 'conditioning' step. The solid line is best fit of equation (*1*) to data from +40 to +80 mV; see text for details. Data from 6 cells. (b) Membrane potential dependence of 'steady state' inactivation of transient outward current. The two-step voltage-clamp protocol used to measure inactivation is shown in inset. Inactivation was measured by the ratio I_T/I_C, where I_T and I_C were current amplitudes in the presence and absence of a conditioning step, respectively. For both controls (○) ($n = 6$) and after quinidine treatment (●) ($n = 6$), the ratio was fitted to a Boltzmann function of the form

$$I_T/I_C = 1/(1 + \exp[(V_m - V_{1/2})/S_{1/2}]$$

See text for values of $V_{1/2}$ and $S_{1/2}$.

−32 ± 1 mV and the slope factor at half-inactivation was 4.2 ± 0.2 mV.

Recovery of transient outward current from quinidine-induced block

The use-dependent behaviour of local anaesthetics during repetitive depolarizations is governed not only by the time course of development of block during depolarization, but also by the amount of recovery from block that occurs in the time intervals between depolarizations. The experiments described below were designed to measure the characteristics of recovery of transient outward current from quinidine-induced block.

Recovery was assessed with a paired pulse voltage-clamp protocol. A 100 ms 'conditioning' step to +50 mV inactivated most of the transient outward current, and the recovery of transient outward current from inactivation at the holding potential (−70 mV) was assessed at different times with a 'test' step (+50 mV, 100 ms). An example of recovery of transient outward current from inactivation is shown in Figure 8a. In control conditions, the transient outward current had almost completely recovered from inactivation when the conditioning-test interval was 140 ms. The time course of recovery from inactivation in controls was fitted to a single exponential function (Figure 8b), with a time constant of 58 ± 4 ms ($n = 9$). In the presence of quinidine, recovery from inactivation was prolonged, with a slow, second phase of recovery following a rapid, initial phase (Figure 8a). The time course of recovery was biexponential (Figure 8b), with 'fast' and 'slow' components with time constants of 59 ± 9 ms and 771 ± 141 ms, respectively ($n = 9$). Figure 8 shows that after a 200 ms interval, the transient outward current had completely recovered from inactivation in control conditions, whereas in the presence of quinidine, substantial block of the transient outward current still remained. This provided the rationale for the 'gapped' voltage-clamp protocols used to measure the time- and voltage-dependence of quinidine block (e.g. Figures 6 and 7).

Discussion

Summary of findings

We have shown that quinidine blocks two components of outward K^+ current in rat isolated ventricular myocytes, a Ca^{2+}-insensitive transient current, and a slowly inactivating delayed rectifier-like component, although only the effects of quinidine on the transient component were examined in

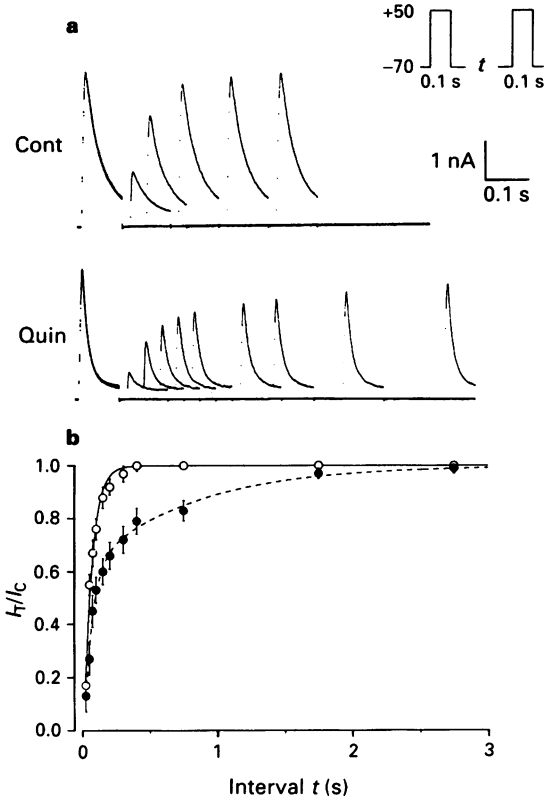

Figure 8 Recovery of transient outward current from quinidine-induced block. (a) A two-pulse protocol was used to measure recovery from inactivation. A 100 ms 'conditioning' step to +50 mV inactivated most of transient outward current; after a variable time t, a 'test' step to +50 mV measured recovery of transient outward current from inactivation. The pulse pairs were applied at 30 s intervals. The currents shown were recorded from the same myocyte in control and in 6 μM quinidine. The solutions contained 10 mM tetraethylammonium chloride. (b) Averaged (mean ± s.e.) recovery data pooled from 9 different cells. 'Test' current amplitude (I_T), normalized to 'conditioning' current amplitude (I_C), is plotted against the time interval between test and conditioning pulses. Control (○; $n = 9$) and after 6 μM quinidine (●). Lines show best-fits of single exponential (Cont) or double exponential (Quin) functions to the data. See text for magnitude of the time constants.

detail. The characteristics of quinidine-induced block of the transient outward current strongly suggested that quinidine blocked open channels, probably from the intracellular side of the membrane. These characteristics included: (*i*) the quinidine-induced increase in the rate of decay of the current, but without significant change in the activation time course (Figures 2 and 4), (*ii*) the use-dependent block (Figure 5), (*iii*) the time course of development of quinidine-induced block paralleled the time course of current (Figure 6), (*iv*) the close correlation between the membrane potential-dependence of current activation and quinidine-induced block (Figure 7), and (*v*) the lack of effect of quinidine on steady-state inactivation of the transient outward current (Figure 7). The implications of these results for models of quinidine-induced block will be discussed in more detail below.

Biophysical analysis of quinidine block of transient outward current

In their simplest form, kinetic models of transient outward current can be written as:

$$[C]_n \rightleftharpoons O \rightleftharpoons I$$

where C, O and I are closed, open and inactivated states of the channel, respectively, and n indicates that there are a series of several closed states leading to an open state (e.g. Zagotta & Aldrich, 1990). At positive membrane potentials, channel 'closure' occurs preferentially by inactivation, hence a prolonged depolarization results in rapid activation of the channel, followed by a slower decay (e.g. Figure 2). This kinetic scheme can be modified to account for block of the open channel by quinidine by adding another pathway:

$$\begin{array}{ccccc} [C]_n & \rightleftharpoons & O & \rightleftharpoons & I \\ & & \downarrow\uparrow & & \\ & & B & & \end{array}$$

where B is a non-conducting, quinidine-blocked channel. This scheme predicts that decay of the current will be accelerated by quinidine, because the open channel can 'close' by two pathways, namely, inactivation ($O \rightleftharpoons I$), and by quinidine block ($O \rightleftharpoons B$). The increase in the rate of decay of the transient outward current in Figures 2 and 4 is consistent with this scheme. In addition, quinidine-induced block cannot occur until the channel opens, which implies that the time course of current activation should be relatively unaffected by quinidine; this result is clearly apparent in the current records in Figure 4.

Quinidine has a positive charge at pH values near 7.2, due to ionization of a tertiary amine group with a pK_a of 8.9. If quinidine blocks transient outward channels by interacting with the open channel, then the block would be expected to be dependent on membrane potential, because the charged ion would interact with the transmembrane electric field in the channel. That this is indeed the case is suggested by the data in Figure 4, which show that the rate of decay of the current in the presence of quinidine is strongly dependent on membrane potential over the range −10 to +50 mV, while it is almost potential-independent in its absence. This suggests that the quinidine blocking step in the kinetic scheme, $O \rightleftharpoons B$, is sensitive to membrane potential. The fact that the rate of decay increased with membrane depolarization is consistent with a positively charged ion moving into the ionic channel from the intracellular side of the membrane. The data in Figure 7a also support this model. These results show that transient outward current activation and block by quinidine increased in parallel with membrane depolarization up to about +30 mV, where this current is fully activated. However, the block produced by quinidine continued to increase at membrane potentials more positive than about +30 mV. This continued increase in block probably reflects the potential dependence of quinidine block of the fully open channel, independent of the effects of membrane potential on channel opening. Assuming this to be the case, the fractional block by quinidine of the transient outward current can be described by the equation (Woodhull, 1973; Snyders *et al.*, 1992)

$$f = [D]/\{[D] + K_d \exp(-\delta F V_m/RT)\} \quad (1)$$

where [D] is the quinidine concentration, K_d is the apparent binding constant (at zero mV membrane potential), and δ is an equivalent electrical distance, i.e., the fraction of the transmembrane potential (V_m) sensed by a single charge at its binding site in the channel. This equation assumes that a single quinidine molecule is required to block a channel (Slawsky & Castle, 1994). Equation (1) was fitted to the data in Figure 7a for membrane potentials from +40 to +80 mV; the best-fit values of δ and K_d were 0.14 and 19.4 μM, respectively. Snyders *et al.* (1992) obtained a similar value of δ (0.19) for quinidine block of a cloned human cardiac delayed rectifier K^+ channel (hKv1.5), expressed in a mouse L cell line.

Comparison with other studies of quinidine-induced block of cardiac K^+ channels

Data from a previous study of the effects of quinidine on Ca^{2+}-independent transient outward current in rabbit atrium (Imaizumi & Giles, 1987) are qualitatively in agreement with

some of the results of the present study. For example, the rate of inactivation of the current was increased by quinidine, the rate of recovery from inactivation was prolonged, and the membrane potential dependence of steady-state inactivation was not affected by quinidine. These similarities suggest that the mechanism of action of quinidine on transient outward current in rabbit atrium and rat ventricle may be identical.

Two studies of the effects of quinidine on rat cardiac transient outward current have appeared recently. Slawsky & Castle (1994) demonstrated many of the features of quinidine-induced block of transient outward current in rat ventricular myocytes shown in the present study, e.g., increased rate of decay of current, use-dependent inhibition, and a slowing of recovery from inactivation in the presence of quinidine, and they interpreted their data in terms of open channel block. Yatani *et al.* (1993) used *Xenopus* oocytes to express a transient outward K^+ channel (RHK1: Kv1.4) cloned from a rat heart cDNA library (Tseng-Crank *et al.*, 1990), and examined the effect of quinidine on macroscopic and single channel currents. Some features of their results were strikingly different from either those in the present study or the findings of Slawsky & Castle (1994); one of the most interesting differences was that quinidine *decreased* the rate of decay of the current, rather than increasing it, as seen in rat myocytes. This effect of quinidine was examined at the single channel level. In control conditions the channels opened in a long, single burst during a membrane depolarization, but after addition of quinidine openings occurred in a series of brief bursts, and the mean open time of the channel during the bursts was reduced by quinidine. Both of these changes in single channel kinetics resulted in the prolongation in time course of inactivation seen in the macroscopic currents. These data were explained by a model in which quinidine blocked the open channel, but the drug was required to dissociate from the channel before it could close during the burst. In addition, Yatani *et al.* (1993) found that the concentration of quinidine required for 50% block of the current was approximately 1 mM, which is more than two orders of magnitude greater than the 50% blocking dose in rat ventricular myocytes (Slawsky & Castle, 1994). There may be several reasons why the effects of quinidine are qualitatively different between these expressed channels and those in the native membrane of rat myocytes. It is not certain that the cloned channel used by Yatani *et al.* (1993) is identical to that found in rat ventricular myocytes, although the rate and potential dependence of inactivation of the cloned channels in control conditions was very similar to that in rat myocytes. It is possible that the native channels are predominantly heteromultimeric, whereas the expressed channels were homomultimeric, since only a single channel clone was injected into the oocytes. It is known that the kinetic and pharmacological properties of heteromultimeric K^+ channels can differ from those of homomultimers (e.g. Isacoff *et al.*, 1990).

Delayed outward K^+ currents are blocked by quinidine in a number of cardiac preparations, including guinea-pig (Hiraoka *et al.*, 1986; Roden *et al.*, 1988; Balser *et al.*, 1991a; Wettwer *et al.*, 1992) and rat (Slawsky & Castle, 1994) ventricle, and rabbit sinoatrial and atrioventricular nodes (Furukawa *et al.*, 1989). In the case of guinea-pig ventricle, quinidine appeared to block multiple components (cf., Sanguinetti & Jurkiewicz, 1990) of delayed rectifier (Balser *et al.*, 1991a; Wettwer *et al.*, 1992). Block by quinidine of the delayed K^+ current in rabbit sinoatrial and atrioventricular nodes (Furukawa *et al.*, 1989), and prolongation of the time constant of tail currents was interpreted in terms of open channel block.

The most comprehensive previous study of the effects of quinidine on a cardiac K^+ channel was carried out by Snyders *et al.* (1992) on a human delayed rectifier channel (hKv1.5) expressed in a stable mouse L cell line. The kinetic and potential-dependent effects of quinidine on this channel were well accounted for by open channel block, in which the quinidine binding site had an equivalent electrical distant of about 20%, referenced to the intracellular side of the membrane. Quinidine produced a rapid phase of 'inactivation' of the current which was qualitatively similar to the effect of quinidine on the time course of the delayed component of outward current in rat ventricular myocytes (cf. Figure 3a).

There are conflicting reports on the effect of quinidine on the inward rectifier K^+ current, I_{K1}. Quinidine was reported to have no significant effect on I_{K1} in rabbit atrium (Imaizumi & Giles, 1987) and Purkinje fibres (Colatsky, 1982), which was in contrast to the results of Salata & Wasserstrom (1988) in canine ventricular myocytes. Balser *et al.* (1991b) reported that quinidine reduced the open probability of single I_{K1} channels recorded from cell-attached patches on guinea-pig ventricular cells, probably by prolonging a closed state of the channels, but these measurements were made at membrane potentials negative to about -100 mV, i.e., far negative to the normal resting potential of the myocytes. The lack of effect of quinidine on the resting membrane potential of rat ventricular myocytes (see Results) is consistent with the study of Slawsky & Castle (1994), which showed that I_{K1} was unaffected even by concentrations of quinidine 100 fold greater than those that blocked transient outward current.

Summary

This study has shown that quinidine is a potent inhibitor of K^+ currents in rat ventricular myocytes, and in the case of Ca^{2+}-independent transient outward current, the mechanism is an open channel block by ionized quinidine. Many other K^+ channels appear to be blocked by quinidine *via* a similar mechanism. This may be expected, given the similarity of the putative ion conducting pore regions, and hence the quinidine binding site, between different K^+ channels (Pongs, 1992).

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