

A Novel Action of Quinine and Quinidine on the Membrane Conductance of Neurons from the Vertebrate Retina

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ABSTRACT The cinchona alkaloids quinine and quinidine have been shown to block a broad range of voltage-gated membrane conductances in a variety of excitable tissues. Using the whole-cell version of the patch clamp technique, we examined the effects of these compounds on voltage-dependent currents from horizontal cells dissociated enzymatically from the all-rod retina of the skate. We report here a novel and unexpected action of quinine and quinidine on isolated horizontal cells. In addition to blocking several of the voltage-activated currents of these cells, the introduction of the alkaloids evoked a large outward current when the cells were held at depolarized potentials. Using tail current analysis, the reversal potential of the outward current was close to 0 mV, and the current was markedly suppressed by extracellularly applied cobalt, acetate, and halothane. Depolarization in the presence of quinine also permitted entry into the cells of extracellularly applied Lucifer yellow (MW = 443 D), whereas a 3-kD fluorescein-dextran complex was excluded. These findings suggest that the large, apparently nonselective conductance induced by quinine and quinidine results from the opening of hemi-gap junctional channels.

INTRODUCTION

The cinchona alkaloid quinine has been used as an antimalarial drug for more than a century (Webster, 1990), and its stereoisomer quinidine is one of the most commonly used agents in the management of cardiac arrhythmias (Ronfeld, 1980; Bigger and Hoffman, 1990). Although questions remain with regard to their precise modes of action, there is abundant evidence that quinine and quinidine are able to suppress ionic conductances through K^+ , Na^+ , and Ca^{2+} channels in the membranes of a variety of different cell types (Yeh and Narahashi, 1976; Wong, 1981; Walden and Speckmann, 1981; Hermann and Gorman, 1984; Hiraoka, Sawada, and Kawano, 1986; Salata and Wasserstrom, 1988; Rae, Dewey, and Rae, 1992). This spectrum of

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activity raised the possibility that these compounds might be of use in exploring the voltage-gated conductances expressed in horizontal cells of the vertebrate retina (Shingai and Christensen, 1983, 1986; Tachibana, 1983; Lasater, 1986; Malchow, Qian, Ripps, and Dowling, 1990). In vivo, these second-order neurons receive input from the photoreceptors, and communicate with each other via a network of electrical (gap) junctions (Naka and Rushton, 1967; Kaneko, 1971; Lamb, 1976; Witkovsky, Owen, and Woodworth, 1983), the type of intercellular coupling that mediates the spread of excitation through cardiac muscle cells (cf. Spray and Bennett, 1985).

In the course of our experiments, we discovered that quinine and quinidine exerted an unusual effect on skate horizontal cells. In addition to their ability to block several voltage-gated ion channels, the drugs opened a large, apparently nonselective conductance when the horizontal-cell membrane was held at depolarizing potentials. We report here the unique properties of that conductance, and its relation to a conductance that may be mediated by the hemi-gap-junctional channels of skate horizontal cells (Malchow et al., 1993). Preliminary findings were published earlier in abstract form (Malchow et al., 1989, 1992).

MATERIALS AND METHODS

Skates (*Raja erinacea* and *R. ocellata*) were anesthetized with 0.1% MS 222 (3-aminobenzoic acid ethyl ester, tricaine) and pithed. The procedure for obtaining isolated horizontal cells and recording transmembrane currents is described in detail in Malchow et al. (1990). Briefly, retinae were dissected free of underlying tissues and incubated in an elasmobranch Ringer solution containing 2 mg/ml papain and 1 mg/ml L-cysteine for 1 h at pH 7.60. Trituration of the retinae through fire-polished Pasteur pipettes yielded isolated cells, which were plated on Falcon 3001 culture dishes containing skate-modified L-15 culture media (Lasater, Dowling, and Ripps, 1984), and kept for periods of up to 1 wk at 14°C. There are two morphologically distinct types of horizontal cell in the all-rod skate retina: one having an extremely large soma is located more distally and is referred to as the external horizontal cell; the other is a slender, more proximally placed element referred to as the internal horizontal cell. The two cell types are readily distinguished in culture, and exhibit similar electrophysiological properties, although hyperpolarizing voltage steps give rise to a more prominent sustained inward current (the inward rectifier) in external horizontal cells (Malchow et al., 1990). In this study, electrical recordings were obtained solely from the large external horizontal cells using the whole-cell configuration of the patch clamp recording technique (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981).

Before recording, dishes were flushed with normal skate Ringer containing (in millimolar): NaCl (250), KCl (6), NaHCO₃ (20), MgCl₂ (1), CaCl₂ (4), NaH₂PO₄ (0.2), urea (360), glucose (10), *N*-2 hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (HEPES) (5), adjusted to pH 7.6 with NaOH. Patch pipettes (~2 μm tip diameter) were filled with a solution containing (in millimolar): KCl (204), CaCl₂ (1), EGTA (11), MgCl₂ (2), MgATP (2), and HEPES (10), pH 7.6 with KOH. When acetate was introduced extracellularly, bicarbonate was omitted from the bathing solution and the pH was adjusted to 7.6; the pipette solution contained neither HEPES nor CaCl₂, and EGTA was added to a final concentration of 0.5 mM. In all experiments, at least 80% of the series resistance was compensated electronically. All drugs were applied extracellularly either by pressure ejection from a pipette (2 μm tip diameter) located ~50 μm from the cell soma, or by bath superfusion through a large-bore pipette (~200 μm tip diameter) located within 1 mm of the cell. Quinine sulfate, quinine HCl, and quinidine HCl were dissolved in

normal Ringer, and adjusted to pH 7.60; these compounds produced qualitatively similar results, and will be considered together. Solutions saturated with halothane were prepared by adding 50 μ l stock halothane to 25 ml Ringer, and then drawing off 20 ml of the solution for use, leaving the undissolved halothane behind. All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO), except for quinine sulfate (Fisher Scientific, Fair Lawn, NJ), halothane (Ayerst Laboratories, New York, NY), 4-aminopyridine (Pfaltz and Bauer Co., Waterbury, CT), and nisoldipine (a generous gift from Dr. Eric A. Schwartz, University of Chicago).

Because of the sensitivity of hemi-gap-junctional channels to changes in the extracellular concentration of free calcium ions (DeVries and Schwartz, 1992; Malchow et al., 1993), it was essential to determine whether the addition of quinine or quinidine altered $[Ca^{2+}]_o$. Measurements were made with an ion-selective vibrating probe using silanized electrodes that carried a calcium ionophore (Fluka Chemical Co., Buchs, Switzerland) in its tip, and were back filled with 100 mM $CaCl_2$ in a 0.5% agar gel (Smith, Sanger, and Jaffe, 1994). Calibration in solutions containing from 0.1 mM to 10 mM Ca^{2+} gave results in accord with the Nernst equation, i.e., ~ 29 mV per decade for divalent cations. Ringer solutions containing 100 μ M or 1 mM of either quinine or quinidine were tested against alkaloid-free Ringers; each paired measurement was repeated at least five times. Analysis of the data showed that, at the concentrations tested, these agents had no detectable effect on $[Ca^{2+}]_o$.

In experiments to determine the approximate size of the channel opened by quinine and quinidine, we examined intracellular fluorescence induced by the influx of Lucifer Yellow (MW 443), carboxyfluorescein (MW 376), and a dextran-fluorescein conjugate (MW 3000, Molecular Probes, Inc., Eugene, OR). Cells were viewed and photographed with a Nikon inverted microscope equipped for incident light fluorescence.

RESULTS

As noted earlier, quinine and quinidine have been shown to block a number of conductances in a variety of cell types. Fig. 1 shows that these compounds exert a similar effect on potassium and sodium conductances of skate horizontal cells. Fig. 1A illustrates the blocking action of 200 μ M quinine on the transient outward current elicited by depolarizing voltage pulses and carried by potassium ions (the "A" current, Conner and Stevens, 1971; Malchow et al., 1990). Both the peak current and the duration of the transient are markedly reduced, suggesting that the compound probably exerts its blocking action on open channels (cf. Wong, 1989). Quinine also reduced the sodium-dependent, TTX-blockable inward current (Fig. 1B), as well as the magnitude of the anomalously rectifying current induced by hyperpolarizing voltage commands (Fig. 1C). In every case, there was considerable recovery of the currents after the chamber was flushed with normal Ringer solution (*right-hand traces*), although the quinine block of the current elicited by hyperpolarization was usually more prolonged and more difficult to reverse. Similar results were obtained in 15 additional cells for each of the three voltage-gated conductances examined under these experimental conditions.

The voltage-gated conductances described above were elicited with voltage steps < 1 s in duration, typical of the protocol often used to characterize the current-voltage relationship of cell membranes. However, when the depolarizing voltage was maintained for tens of seconds, an additional conductance was clearly visible (cf. Malchow et al., 1993). The top trace in Fig. 2 shows the current elicited from an external horizontal cell in normal Ringer by a 100-ms voltage step (*top trace*) from

–70 to +40 mV. In response to the brief voltage pulse, the most prominent feature of the response was the large transient A current, which decayed rapidly to a smaller sustained outward current. However, when the cell was depolarized to +50 mV and held at this potential for a substantially longer period of time (i.e., 16 s), a time-dependent increase in the outward current was seen. There is good evidence that this small, slowly developing outward current, which we have previously dubbed the “Q” current, is associated with the opening of hemi-gap-junctional channels on the rod-driven horizontal cells of skate (Qian, Malchow, and Ripps, 1989; Malchow et al., 1992, 1993). An outward current observed under conditions of low external

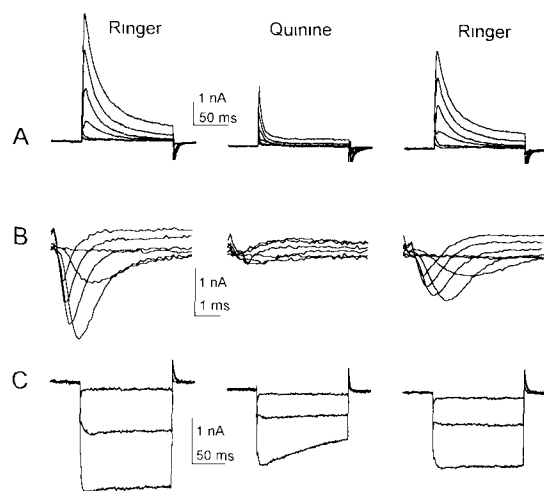


FIGURE 1. Block of voltage-activated currents by quinine. Shown are currents before drug application (*left*), during superfusion of 200 μ M quinine (*middle*), and after washout with Ringer (*right*). (*A*) Currents elicited in an external horizontal cell by 200-ms voltage steps ranging from –60 to +40 mV in 20-mV increments; holding potential was –70 mV. Depolarizations induced a transient outward current (A current) which reached several nanoamps in amplitude. The addition of quinine to the bath led to a marked reduction in the

magnitude and duration of the transient current. The block was partially reversed by superfusion with normal Ringer. Note that this cell did not display a transient inward current at the beginning of the voltage pulse (see below). (*B*) Brief depolarizing pulses from –60 to +40 mV (initial holding potential –70 mV) induced a fast inward current (the transient outward current was blocked in this instance with 10 mM 4-aminopyridine). The transient inward current is sensitive to TTX and is carried by sodium (Malchow et al., 1990). Addition of quinine markedly reduced the size of this current (*middle*), and again the effect of quinine was partially reversed upon washout of the quinine. (*C*) Hyperpolarizing steps (–120 to –80 mV from a holding potential of –70 mV) induced a large inward current in skate horizontal cells (*bottom left*). The application of 200 μ M quinine resulted in a reduction in this current.

calcium in cone-driven catfish horizontal cells has also been identified with the opening of hemi-gap-junctional channels, and has been termed I_γ by DeVries and Schwartz (1992). In addition, a current with similar properties has been recorded in *Xenopus* oocytes expressing a gap-junctional protein (cx46) cloned from rat lens (Ebihara and Steiner, 1993). Further study will be necessary to determine the degree to which these conductances are equivalent.

To examine the effects of quinine on the magnitude and time course of the Q current, the response shown at the bottom of Fig. 2 has been replotted in the top trace of Fig. 3 *A* at a reduced gain (making the Q current difficult to discern). Shown

at the same gain in the middle trace of Fig. 3 *A* is the effect of the depolarizing pulse on the same cell after superfusing it for 2 min with 100 μ M quinine. Note that the fast transient outward current was again suppressed, but a much larger, more rapidly developing outward current is now evident. The effect of quinine was reversible, as shown by the lowest trace of Fig. 3 *A*; washout of the quinine with normal Ringer's solution led to a marked decrease in the Q current, as well as a partial return of the A current.

The outward current activated by depolarization in the presence of quinidine or quinine was associated with a conductance increase. The top trace in Fig. 3 *B* shows current recordings in response to brief 20-mV hyperpolarizing voltage pulses applied before, during, and after a prolonged (20 s) depolarizing voltage step from -70 mV to $+50$ mV. With the cell held at -70 mV, the 20-mV pulses evoked large inward currents, reflecting the movement of ions through the anomalously rectifying

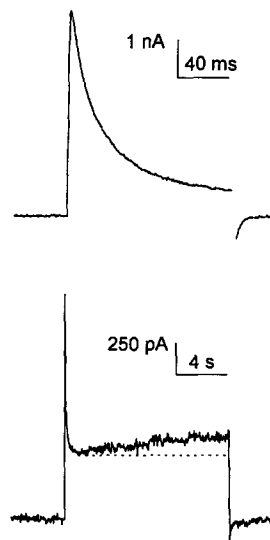


FIGURE 2. Prolonged depolarizations promote the development of a slow outward current in isolated skate horizontal cells. The top trace shows the current elicited from an external horizontal cell in response to a 100-ms voltage step from -70 to $+40$ mV. This voltage pulse elicited a large, transient outward current that rapidly decayed towards an apparently steady level. The lower trace shows, at a higher gain, and with a longer depolarizing voltage pulse (this time to $+50$ mV), that a slowly developing outward current can be detected in the same cell; this current has been dubbed the Q current (Malchow et al., 1993).

conductance. Jumping the voltage to $+50$ mV activated the transient outward current; this was followed by a sustained outward current, during which time the currents induced by the 20-mV hyperpolarizing voltage pulses were relatively stable but considerably smaller. When the bath solution was switched to one containing 200 μ M quinine (Fig. 3 *B*, bottom trace), the currents induced initially by the voltage pulses were smaller than those obtained in Ringer's, a further indication that quinine acts to partially reduce the current flowing through the anomalous rectifier (see Fig. 1 *C*). Moreover, stepping the voltage to $+50$ mV no longer evoked the transient A current, but activated the slow, large outward current; the latter was accompanied by an increase in the current associated with the 20-mV voltage steps, indicating a significant increase in conductance.

Fig. 4 compares currents elicited in Ringer's solution with those obtained after superfusion with 200 μ M quinine. As shown in Fig. 4 *A*, the Q current became

apparent at potentials more positive than 0 mV and increased with time throughout the 10-s voltage step. Superfusion of 200 μM quinine resulted in a marked potentiation of the currents at potentials > 0 mV, but had little effect on currents elicited by voltage steps ranging from -50 to 0 mV (Fig. 4 B). The responses did not appear to reach a final steady peak during the 10-s depolarization, and attempts to hold the cells at high positive potentials for appreciably longer periods of time often resulted in leakage of the seal and disruption of the whole-cell configuration (see also comments by Ebihara and Steiner, 1993). The current-voltage relations of the Q current and the current elicited by quinine are shown in Fig. 4 C. The data suggest

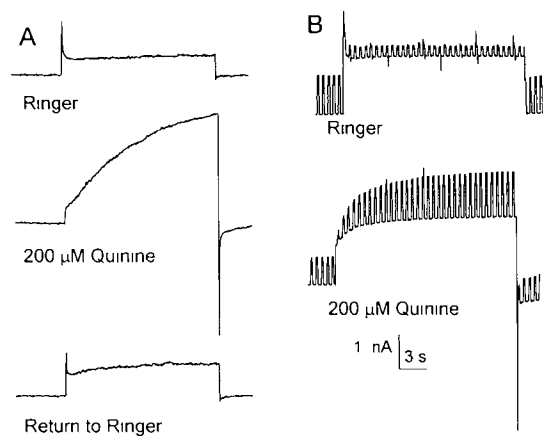


FIGURE 3. Effects of quinine on isolated external horizontal cells from the skate retina. Currents were elicited by 16-s voltage steps from a holding potential of -70 to $+50$ mV. (A) The upper trace is a replot at lower gain of the recording shown in the lower portion of Fig. 2. The middle trace shows the response after the cell had been bathed for 2 min in Ringer containing 200 μM quinine sulfate. The transient outward current was abolished, and a large, slow outward current

was evident. Repolarization to -70 mV typically produced a large inward tail current that turned off rapidly. The bottom trace, recorded after 2 min in normal Ringer, shows that the effects of quinine were reversible. (B) The quinine-induced outward current is associated with a large conductance increase. Responses to a voltage step from -70 to $+50$ mV were obtained with the cell in normal Ringer (*top trace*) and after superfusion for 2 min in 100 μM quinine (*bottom trace*); brief 20-mV hyperpolarizing pulses were superimposed upon the longer voltage steps to examine changes in conductance. With the cell held at -70 mV, the currents elicited by the 20-mV pulses were larger in Ringer solution than with the cell bathed in quinine, suggesting that the drug reduced the conductance through the anomalous rectifier. When the voltage was stepped to $+50$ mV, however, the currents elicited by the 20-mV pulses were much larger in the presence of quinine, indicating a large increase in conductance associated with the development of the quinine-induced outward current.

that quinine enhanced the magnitude of the Q current without causing a significant shift of the curve along the voltage axis; this is more evident after replotting the data as a normalized function in which the peak currents at $+40$ mV in Ringer and quinine were set to a value of 1 (Fig. 4 C, *inset*). Thus, quinine did not appear to alter the voltage-activation curve of the Q current.

The enhancement of the Q current as a function of the concentration of quinine is shown in Fig. 5. The top set of traces of Fig. 5 A show the responses of a single cell to a voltage jump first to -40 (to inactivate the transient outward current) and then to $+30$ mV for 10 s; this protocol was repeated six times, with a 1.5-min interval

between each voltage step. With the cell bathed in Ringer's supplemented with 0.5 mM tetrodotoxin, 15 mM tetraethylammonium, and 10 μ M nifedipine, only a slight increase in the outward current at the end of the 9.5-s voltage step was seen. The bottom set of traces show responses from another horizontal cell to the same voltage protocol first in Ringer's, and then after superfusing the cell with 20, 50, 200, 500, and 1,000 μ M quinine. The transient outward current was abolished by quinine, and

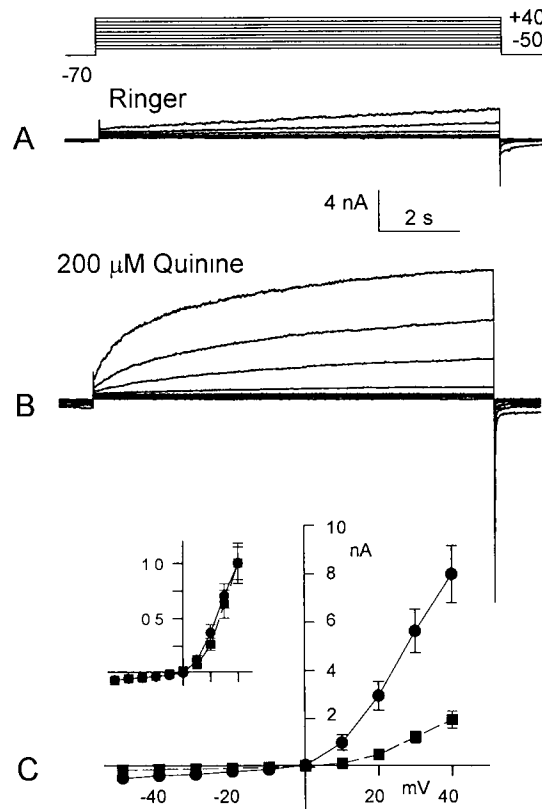


FIGURE 4. Current-voltage relation for the quinine-induced current. (A) Responses of a cell to 10-s voltage jumps ranging from -50 to $+40$ mV from a holding potential of -70 mV; the voltage protocol is shown at the top of the figure. The cell was bathed in a Ringer solution containing 0.5 μ M tetrodotoxin, 10 μ M nifedipine, 15 mM TEA, 10 mM 4-aminopyridine and 10 mM cesium chloride to reduce the contribution from other voltage-gated conductances. 30-s intervals were allowed between voltage steps of -50 through -10 mV, while 1-min intervals separated voltage steps from 0 to $+40$ mV; cells were held at -70 mV during the interstimulus interval. (B) Responses from the same cell to the identical voltage protocol when superfused with the Ringer solution described plus 200 μ M quinine. Note the development of a large outward current at positive potentials, and a large tail current upon repolarization to -70 mV. (C) Plot of the I - V relation in Ringer (squares) and 200 μ M quinine (circles). Data points show the mean currents (\pm the standard error) as measured during the last 120 ms of each voltage jump; points displaying no error bars had variances smaller than the plotted point. The inset shows a replot of the same data normalized to equate the values obtained at $+40$ mV for both Ringer and 200 μ M quinine.

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the Q current grew as the quinine concentration was increased. In this case, the peak current induced at the end of the $+30$ -mV voltage step in 500 μ M quinine was approximately the same amplitude as that produced in 1 mM quinine, and at these high concentrations a plateau seemed to be reached. Also worth noting is the slight increase in the baseline current seen at -70 mV when the cell was superfused with 1 mM quinine. In cells superfused with 2 mM quinine (data not shown), the baseline

current at -70 increased substantially; we do not yet know whether this inward current was distinct from the effects of quinine on the Q current. Fig. 5 *B* shows a semilogarithmic plot of the amplitude of the current obtained at the end of the $+30$ -mV voltage step as a function of the concentration of quinine; the point at 0 on the concentration axis indicates the Q current that was present when no quinine was added. Caution should be exercised in interpreting this graph, since at low quinine concentrations, the responses did not reach a steady state when the $+30$ mV voltage step was terminated.

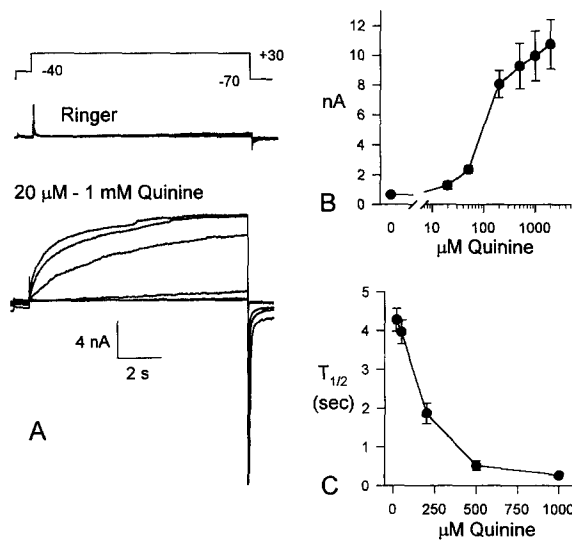


FIGURE 5. Dose dependence of the outward current elicited by quinine. (A) The top trace shows the voltage protocol: cells were first held at -40 mV for 725 ms to reduce the size of the transient outward current, and then jumped to $+30$ mV for 9.51 s. The solution was then switched and 1.5 min allowed to elapse before beginning another episode; control experiments indicated that the effects of the drug were maximal after this interval. The middle traces show responses from one horizontal cell to six such voltage jumps with the cell bathed in Ringer solution con-

taining 0.5μ M tetrodotoxin, 10μ M nifedipine, and 15 mM tetraethylammonium to reduce sodium, potassium, and calcium conductances. The bottom traces show responses from another cell to the same voltage protocol when superfused with Ringer, and then with 20, 50, 200, 500, and 1,000 μ M quinine. (B) Plot of current vs the concentration of quinine. The mean current during the last 120 ms of the $+30$ mV pulse was determined for each quinine concentration; the averaged data from six cells (\pm SEM) are shown (points lacking error bars had errors smaller than the size of the plotted point). (C) Time dependence of the outward current as a function of the concentration of quinine. The time required to reach one half of the peak value at the end of the $+30$ mV voltage jump for each concentration of quinine was determined; plotted points are the average values obtained from six cells.

Raising the concentration of quinine led also to an increase in the rate of development of the Q current. The data of Fig. 5 *C* plots the time required to reach one half the peak amplitude ($T_{1/2}$) as a function of concentration. Owing to the complex kinetics of the current responses, the $T_{1/2}$ value was used as an index of the rise time of the response. Although the increase in current at 200 μ M quinine could be reasonably well described by a single exponential, the currents elicited at higher concentrations appeared to be better fit by the sum of two or three exponentials, whereas the currents at 20 and 50 μ M quinine could not be characterized by the sum

of exponentials. Nevertheless, the fact that the $T_{1/2}$ values decrease as the quinine concentration is increased suggests that quinine induces its effect by altering the kinetics of the Q current.

The notion that the cinchona alkaloids enhance the depolarization-induced outward current (i.e., the Q current) associated with gap-junctional activity was supported by a series of pharmacological, electrophysiological, and dye transfer experiments. For example, the current induced by quinine and quinidine was partially antagonized by several agents known to impede ionic currents through gap-junctional channels (DeVries and Schwartz, 1989; Qian, Malchow, and Ripps, 1993). As illustrated in Fig. 6, the addition of cobalt, halothane, or acetate to the bath

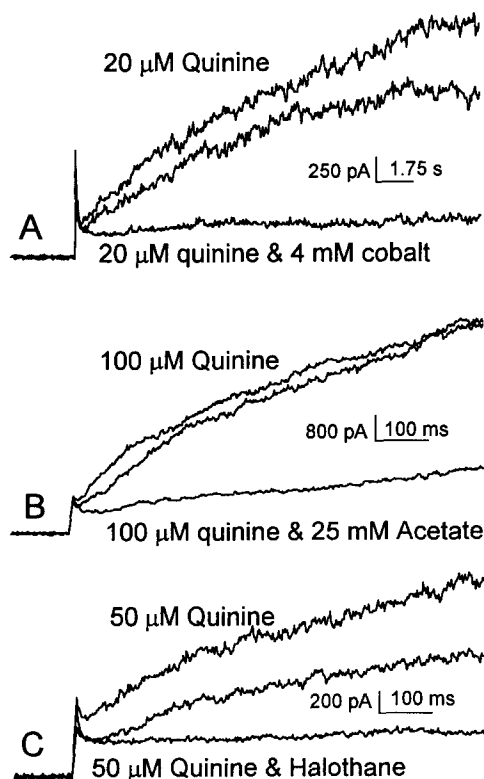


FIGURE 6. Suppression of the quinine-induced current by cobalt, halothane, and acetate. (A) Reversible block by 4 mM cobalt with the cell bathed continuously in 10 μM quinine. Current recordings obtained in response to a voltage step from -70 to $+50$ mV before, during, and after the addition of cobalt. A relatively small transient outward current persists, due probably to the low concentration of quinine used in this experiment. In this and in B and C of the figure, the upper trace illustrates the initial response to quinine, the lower trace shows the effect of the blocking agent, and the central trace represents partial recovery of the response after 2 min washout of the blocker. (B) Reduction of the quinine-induced current by application of 25 mM acetate. Note that the acetate solution was adjusted to pH 7.6. (C) Block of the quinidine-induced current by bathing the cell in a Ringer solution saturated with halothane.

solution markedly suppressed the quinine-induced current, and in each case, the block was partially or completely reversible. Although the modes of action of these agents are not well understood, the addition of acetate was shown previously to be effective in acidifying the interior of horizontal cells and reducing current flow between pairs of electrically-coupled cells (DeVries and Schwartz, 1989; Qian et al., 1993) as well as through hemi-gap-junctional channels on individual cells (DeVries and Schwartz, 1992; Malchow et al., 1993).

If the current induced by the cinchona alkaloids reflects the opening of hemi-gap-junctional channels, then it should display a relatively low degree of ionic selectivity,

and consequently, a reversal potential near 0 mV. Fig. 7A shows tail current recordings from a horizontal cell stepped to +50 mV for 10 s and then jumped decrementally to a series of more negative potentials. The cell was bathed in 100 μ M quinidine; the Ringer solution also contained 10 mM TEA and 6.5 μ M nisoldipine in the bath solution (to reduce contributions from the delayed rectifier and the L type calcium conductance, respectively), and the holding potential of -40 mV suppressed the transient sodium and potassium conductances activated by depolarizing pulses. A plot of the mean amplitude of the tail currents from four cells recorded with this protocol is shown in Fig. 7B. Note that (a) the instantaneous conductance appears to be linear over the range of -50 to +50 mV, and (b) the reversal potential was very close to 0 (0.9 ± 4 mV). Tail currents from six other cells bathed in 100 μ M quinidine had reversal potentials of $+6.1 \pm 1.6$ mV.

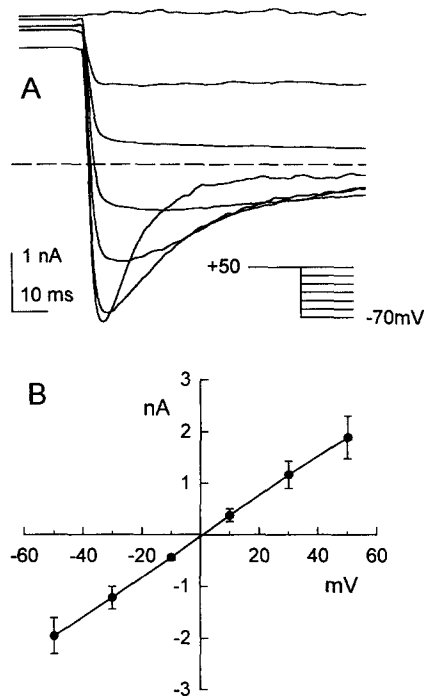


FIGURE 7. (A) Quinidine-induced tail currents elicited by repolarizing steps from a potential of +50 mV applied for 10 s. Responses were obtained first with the cell in Ringer, and then after it had been bathed in 100 μ M quinidine; the traces shown represent the algebraic difference between the two current recordings, i.e., the currents due to quinidine alone. (B) Plot of the mean peak amplitude of the tail current as a function of the repolarization voltage. The reversal potential of 0.9 mV (± 4 mV, $n = 4$) suggests a nonspecific ionic channel. Error bars are standard errors.

The tail currents shown in Fig. 7A indicate that the inactivation of the quinidine-induced current occurred quite rapidly upon repolarization. At potentials more negative than -10 mV, the decay tended to follow an exponential time course, although repolarization to -70 mV often was better fit with the sum of two exponentials. The time constant appeared to decrease as potentials became more negative. The mean value for the time constant from four cells bathed in quinidine was 27.5 ± 10 ms at -30 mV, 15.5 ± 3 ms at -50 mV, and 6.7 ± 3 ms for the faster process recorded at -70 mV.

Under the present experimental conditions, the chloride equilibrium potential (~ -5 mV) was not far removed from the experimentally determined reversal

potential, thus raising the possibility that chloride might serve as the main current carrier. Experiments in which all of the extracellular chloride was replaced with isethionate, produced little change in the observed currents, and no significant shift in the reversal potential. If the junctional channels are impermeant to isethionate, its lack of effect on the reversal potential would suggest that the quinine-induced currents are not carried by chloride. However, it is important to consider the likelihood that isethionate (MW 126 D) is also permeant, and that the conductance does not distinguish between chloride and isethionate. We have not yet examined

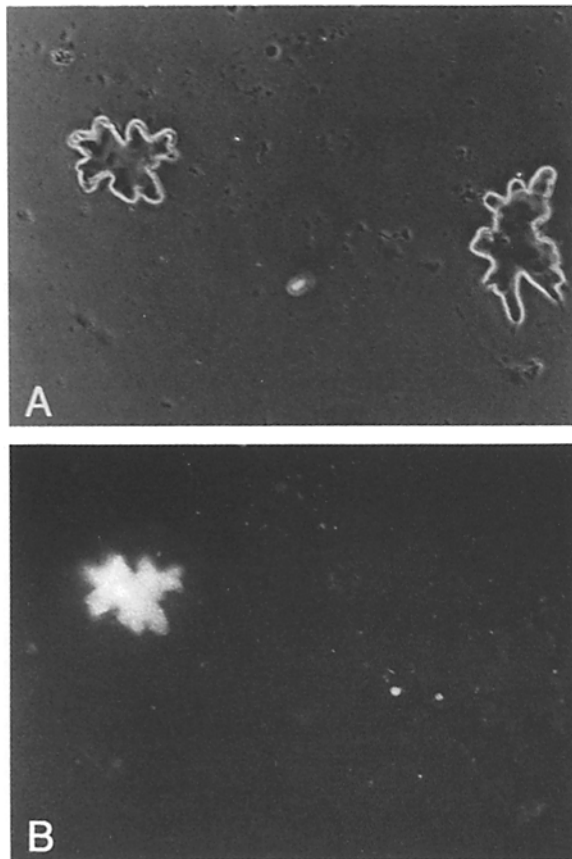


FIGURE 8. (A) Phase micrograph of two isolated external horizontal cells from the skate retina. Both cells were bathed in a Ringer solution containing $100 \mu\text{M}$ quinine and 10 mM Lucifer yellow. The cell on the left was whole-cell clamped, and alternately jumped from -70 mV to $+50 \text{ mV}$ in a 3-s duty cycle for a total of 8 min. The bath solution was then flushed thoroughly to remove extracellular quinine and Lucifer, and the cells were examined using fluorescence microscopy. (B) Fluorescence due to the accumulation of Lucifer yellow was seen in the cell that had been depolarized in the presence of quinine, but not in the cell that had not been depolarized. Dye incorporation was seen in six other cells that had been subjected to the depolarization/quinine protocol.

these alternatives, nor have we attempted to explore fully the permeability characteristics of the channel. Nevertheless, the data are consistent with the notion that the conductance increase is associated with a nonselective ion channel, a property characteristic of gap-junctional channels (Spray and Bennett, 1985).

Further evidence suggesting that quinine and quinidine promote the opening of large nonselective ionic channels was obtained by examining the ability of fluorescent dyes to permeate into the cells. Fig. 8A shows a phase micrograph of two external horizontal cells bathed in a Ringer containing $100 \mu\text{M}$ quinine and 10 mM Lucifer

yellow. The cell on the left was whole-cell clamped, and the voltage held at -70 mV for 3 s, then stepped to $+50$ mV for 3 s; this alternating sequence was repeated for 8 min. Only the cell that had been depolarized in the presence of quinine incorporated the fluorescent dye (Fig. 8 B). In control experiments, cells that were whole-cell clamped, bathed in 100 μ M quinine, but not depolarized (i.e., held at -70 mV for 8 min), did not display uptake of Lucifer. Furthermore, cells that were depolarized with the above protocol in the absence of quinine showed no detectable labeling. Thus, intracellular incorporation of the dye required both depolarization and the addition of quinine. Finally, the same protocol was followed in three experiments in which the

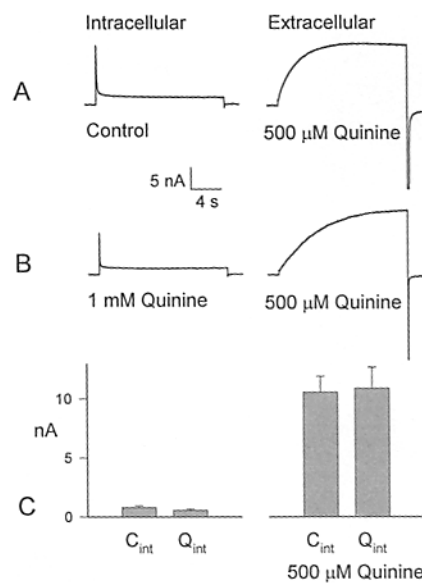


FIGURE 9. Effects of intracellular vs extracellular quinine. (A) The current recorded from one cell in response to a voltage step from -70 to $+30$ mV; the depolarizing voltage was maintained for 17 s. The response was obtained 5 min after establishing a whole-cell configuration using standard intra- and extracellular solutions. The trace at the right shows the response of the cell to the same voltage protocol 45 s after superfusion with 500 μ M quinine; the transient outward current has been abolished, and the large quinine-induced outward current is evident. (B) The response from another cell in which the intracellular solution contained 1 mM quinine. The left trace was obtained 5 min after establishing a whole-cell recording; the transient outward current is again evident, but there is no indication of a quinine effect on the sustained outward current. The right trace shows that extracellular application of 500 μ M quinine again blocked the A current and induced the large, slowly developing outward current in the same cell. (C) Bar graphs comparing the magnitudes of the outward currents in response to intracellular (left) and extracellular (right) applications of quinine. Results represent mean values for five cells with normal intracellular solution (C_{int}) and seven cells in which the intracellular solution was supplemented with 1 mM quinine (Q_{int}); error bars are standard errors. Note that quinine was effective only when applied extracellularly.

current. The right trace shows that extracellular application of 500 μ M quinine again blocked the A current and induced the large, slowly developing outward current in the same cell. (C) Bar graphs comparing the magnitudes of the outward currents in response to intracellular (left) and extracellular (right) applications of quinine. Results represent mean values for five cells with normal intracellular solution (C_{int}) and seven cells in which the intracellular solution was supplemented with 1 mM quinine (Q_{int}); error bars are standard errors. Note that quinine was effective only when applied extracellularly.

cells were bathed in a Ringer solution containing a 3000 MW dextran-fluorescein complex; no fluorescence was observed with this relatively large molecular weight compound, although in three experiments, carboxyfluorescein itself, like Lucifer yellow, readily entered the cell. These findings are a good indication that the conductance opened by quinine and quinidine is not due simply to a rupture of the cell membrane.

Experiments in which quinine was applied extracellularly or introduced intracellularly via the patch pipette suggest that the drug exerts its effect on the external face of the hemi-gap-junctional channel. Fig. 9 presents the results of these studies. The

left trace of Fig. 9A shows the response of one cell to a 10-s voltage step to +30 mV recorded 5 min after the establishment of the whole-cell configuration using the normal intracellular solution; the transient outward current is prominent, and the outward current at the end of the voltage step is relatively flat. The left trace of Fig. 9B shows the response of a different cell to the same voltage pulse 5 min after rupturing the patch using an internal solution containing 1 mM quinine. The transient current was still evident, and the outward current elicited at the end of the +30 mV step did not appear significantly different from that obtained in control cells. In both cases, the cells were still sensitive to 500 μ M quinine applied externally, as indicated in the right-most set of traces.

Fig. 9C shows a bar graph of the amplitude of the currents elicited at the end of the voltage step. At the left are shown the values obtained 5 min after the establishment of the whole-cell mode using the standard intracellular solution (C_{int} , five cells), and for seven cells in which the internal solution contained 1 mM quinine in addition to the normal constituents (Q_{int}). Clearly, intracellular quinine did not have a significant effect on the magnitude of the Q current recorded 5 min after establishing the whole-cell configuration. Although it was not possible to directly measure the degree to which the quinine diffused into the cell, comparable experiments with Lucifer yellow in the pipette solution showed intense fluorescence at 5 min into the whole-cell recording.

The graphs on the right of Fig. 9C show the average responses from the same group of cells to externally applied 500 μ M quinine. Note that irrespective of the intracellular solution, (a) the cells remained responsive to extracellularly applied quinine, and (b) the size of the currents did not appear to be significantly different. These data provide a good indication that the effects of quinine were exerted on the external face of the cell.

DISCUSSION

The principal finding of this study concerns the novel action of quinine and quinidine on skate horizontal cells. Although these drugs blocked voltage-sensitive potassium and sodium conductances present in horizontal cells (Fig. 1), we found that the cinchona alkaloids induced a large, relatively slow, outward current when the cells were depolarized beyond 0 mV. Analysis of this current suggests that it represents the enhancement by quinine and quinidine of the depolarization-induced Q current, a current that is thought to reflect the opening of hemi-gap-junctional channels on isolated horizontal cells (Qian et al., 1989; Malchow et al., 1993). The results of a series of experiments on the current-voltage relationship, pharmacology, and channel properties of this unique conductance are consistent with this view. For example, when applied extracellularly, quinine and quinidine caused a large *increase* in the magnitude and rate of growth of the Q current (Figs. 2 and 3). Moreover, like the Q current, the drug-induced current showed a reversal potential near 0 mV, and it was suppressed by extracellularly applied cobalt, acetate, and halothane (Fig. 6). Furthermore, depolarization in the presence of quinine also permitted entry into the cells of extracellularly applied Lucifer yellow (MW 443), whereas a 3-kD fluorescein-dextran complex was excluded (Fig. 8). These findings lend support to the notion that the large, apparently nonselective conductance induced by quinine and quinidine results

from the opening of hemi-gap-junctional channels presumed to be present in the membranes of isolated horizontal cells of fish retinae (DeVries and Schwartz, 1992; Malchow et al., 1993).

The mechanism by which the cinchona alkaloids promote the opening of hemi-gap-junctional channels in skate horizontal cells has yet to be elucidated. Although the hemi-gap-junctional channels of fish horizontal cells can be opened by lowering the concentration of extracellular calcium (DeVries and Schwartz, 1992; Malchow et al., 1993), the effects of the cinchona alkaloids do not seem to be due to a fall in $[Ca^{2+}]_o$; measurements with calcium-sensitive electrodes showed no decrease in calcium concentration when 100–1,000 μ M quinine or quinidine was added to the Ringer (see Materials and Methods). Nor is it likely that these drugs act by altering external pH; the pH of the quinine solution was adjusted to match that of the control Ringer (7.6) before each experimental run.

The question of whether quinine and quinidine affect internal pH or the intracellular concentration of calcium remains to be determined. However, it appears unlikely that an increase in pH_i or a decrease in $[Ca^{2+}]_i$ is responsible for the alkaloid-induced currents. Although it is known that gap junctional conductance is sensitive to alterations in internal pH (cf. Spray, Harris, and Bennett, 1981), the patch pipettes contained 10 mM HEPES, which should provide adequate control of internal pH. As for the possibility that quinine induces a reduction in $[Ca^{2+}]_i$, there is good evidence that a decrease in calcium shifts the steady state voltage-activation curve of gap-junctional channels to more negative potentials (Obaid, Socolar, and Rose, 1983); no such shift was induced by the cinchona alkaloids (Fig. 4), suggesting a different mode of action. Nevertheless, these issues can be addressed by using fluorescent probes to monitor the changes in intracellular pH and calcium induced by the application of quinine and quinidine, and experiments of this type are currently being undertaken in our laboratory. Alternatively, quinine and quinidine may interact with a receptor linked to a second messenger system, or directly with proteins (connexins) forming the hemi-junctional channel—possibly on the external face—to promote the opening of the channel when the cell is depolarized.

As mentioned earlier, it is well known that quinine and quinidine can have profound effects on the properties of cardiac cells, and quinidine in particular has remained an important tool in the clinical management of ventricular arrhythmias (Bigger and Hoffman, 1990). The therapeutic action of this compound has been ascribed to its ability to block sodium and potassium conductances, resulting presumably in a prolongation of the action potential, but the issue has yet to be fully resolved (Hiraoka et al., 1986; Prinzmetal, Ishikawa, Oishi, Ozkan, Wakayama, and Baines, 1967; Mirro, Watanabe, and Bailey, 1981; Colatsky, 1982). Based on the results of this study, it is interesting to speculate as to some alternative modes of action. For example, if hemi-gap-junctional channels are present on cardiac myocytes, quinidine might exert its effect by the depolarization induced by the opening of these nonselective channels. On the other hand, if quinidine were to promote the opening of the gap-junctional channels that mediate the synchronous activity of cardiac muscle cells, it is possible that the stabilizing effects of the drug on heart rate result in part from its ability to enhance intercellular communication between myocytes. However, it is important to stress that we have not examined the effects of

quinidine on cardiac muscle cells, nor have we studied the effects of these drugs on the conductances of intact gap junctions of electrically coupled nerve or muscle cells. Moreover, the gap-junctional proteins in mammalian heart cells are probably very different from those in skate horizontal cells, and it is unlikely that they exhibit identical pharmacological properties. Nevertheless, further study of the effects of quinidine on the electrical junctions between cardiac myocytes is clearly warranted.

In sum, our results indicate that new approaches to the study of electrical synapses could be attempted using pharmacological agents to probe the properties of the gap-junctional proteins on individual cells, and thus, define more precisely the molecular mechanisms responsible for modulating gap-junctional communication.

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