Quinine blocks specific gap junction channel subtypes

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We demonstrate that the antimalarial drug quinine specifically reduces currents through gap junctions formed by some connexins (Cx) in transfected mammalian cells, but does not affect other gap junction types. Quinine blocked Cx36 and Cx50 junctional currents in a reversible and concentration-dependent manner with half maximal blocking concentrations of 32 and 73 μM, respectively; Hill coefficients for block by quinine were about 2 for both connexins. In contrast, quinine did not substantially block gap junction channels formed by Cx26, Cx32, Cx40, and Cx43, and only moderately affected Cx45 junctions. To determine the location of the binding site of quinine (pKa = 8.7), we investigated the effect of quinine at various external and internal pH values and the effect of a permanently charged quaternary derivative of quinine. Our results indicate that the binding site for quinine is intracellular, possibly within the pore. Single-channel studies indicated that exposure to quinine induced slow transitions between open and fully closed states that decreased open probability of the channel. Quinine thus offers a potentially useful method to block certain types of gap junction channels, including those between neurons that are formed by Cx36. Moreover, quinine derivatives that are excluded from other types of membrane channels may provide molecules with connexin-specific as well as connexin-selective blocking activity.

Gap junction channels of vertebrates are formed of a family of proteins known as connexins (Cx) that are expressed in an overlapping pattern of tissue distribution (1). These channels provide pathways of intercellular communication through which pass ions and small molecules up to 1 kDa in mass or 10–16 Å in diameter. The functional roles of gap junctions formed of individual connexins have been illuminated by recent studies on connexin-deficient mice and in both humans and rodents harboring dysfunctional connexin mutations (2, 3). In addition to their roles in normal tissue function, gap junctions may also be deleterious in pathological situations, providing a pathway for spread of cellular injury (4). Because gap junction blockade may prevent such spread, it is desirable to identify agents selective for gap junction channels and in particular those agents whose action is connexin-specific.

Gap junction channels formed by various connexins can be closed by a number of factors, including intracellular acidification (5), transjunctional voltage (6), long-chain alcohols (i.e., heptanol and octanol; ref. 7), halothane and ethrane (8), glycyrrhetinic acid derivatives (9), oleamide and derivatives (10), and arachidonic acid or its metabolites (11). Sensitivity of gap junctions to low pH and to transjunctional voltage depends on the connexin subtype. For example, closure of Cx45 gap junctions is strongly voltage-dependent, whereas voltage dependence of Cx36 channels is quite weak (12–14). Similarly, closure induced by acidification yields apparent pKa values ranging from about 7.0 for Cx50 to 6.0 for Cx32 (see ref. 15). Although only a few studies have examined the connexin specificity of other uncoupling agents, differences thus far demonstrated have been quite modest (e.g., the difference in the EC_{50} values for closure of Cx40 and Cx43 channels by halothane was less than 2-fold; ref. 16).

To extend the search for uncoupling agents that might possess greater specificity for individual connexin types, we have investigated the effects of quinine, an antimalarial drug. Previous studies indicated that quinine activated putative hemichannel-like currents in horizontal cells of skate and perch retina (17–19) and potentiated gap junction hemichannel currents in oocytes expressing certain connexins (e.g., Cx35) and not others (e.g., Cx43) (20). We now demonstrate in mammalian cells that quinine blocks certain types of gap junction channels in a reversible and concentration-dependent manner, whereas gap junctions formed of other connexins are unaffected. In addition, our results suggest that this action of quinine is mediated through binding to an internal site in the gap junction channel.

Methods

DNA Construction and Transfection. Most of the electrophysiological experiments described here were performed on N2A cells that were either stably transfected with connexins or transiently cotransfected with connexin and enhanced green fluorescent protein cDNAs in separate vectors as described (21). In some experiments involving Cx36 and Cx43, RIN cells (generously provided by P. Meda, University of Geneva, Switzerland) were also used to demonstrate that the effect of quinine was not cell-type specific. The connexins used in the study were hCx32, rCx36, rCx40, rCx43, mCx45, and mCx50 (where h, r, and m refer to human, rat, and mouse cDNAs) and the Cx36 fish homologues, Cx35 and Cx34.7 (cDNAs for Cx35 and Cx34.7 generously provided by Thomas White, State University of New York, Stony Brook). Transiently transfected cells were dissociated at 8–12 h after transfection and plated at low density on 1-cm round glass coverslips.

Electrophysiology. Junctional conductance was measured between cell pairs by using the dual whole cell voltage clamp technique with Axopatch 1C or 1D patch-clamp amplifiers (Axon Instruments, Foster City, CA) at room temperature. The solution bathing the cells contained 140 mM NaCl, 5 mM KCl, 2 mM CsCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Hepes, 5 mM dextrose, 2 mM pyruvate, and 1 mM $BaCl_2$, pH 7.4. Patch electrodes had resistances of 3–5 MΩ when filled with internal solution containing 130 mM CsCl, 10 mM EGTA, 0.5 mM $CaCl_2$, 3 mM MgATP, 2 mM Na_2ATP, and 10 mM Hepes, pH 7.2. Macroscopic and single-channel recordings were filtered at 0.2–0.5 kHz and sampled at 1–2 kHz. Data were acquired by using PCLAMP6 or PCLAMP8 software (Axon Instruments); analysis was performed with PCLAMP8 and ORIGIN 6.0 software (Microcal Software, Northampton, MA).

Each cell of a pair was initially held at a common holding potential of 0 mV. To evaluate junctional coupling, 200-msec hyperpolarizing pulses from the holding potential of 0 mV to −10 mV were applied to one cell to establish a transjunctional voltage gradient (V_j), and junctional current was measured in the second cell (held at 0 mV). Single-channel currents were investigated in weakly coupled cell pairs (1 or 2 channels) without the use of uncoupling agents by applying −20-mV pulses to one cell

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Abbreviations: pH_i, internal pH; pH_e, external pH; BQ, *N*-benzylquininium.

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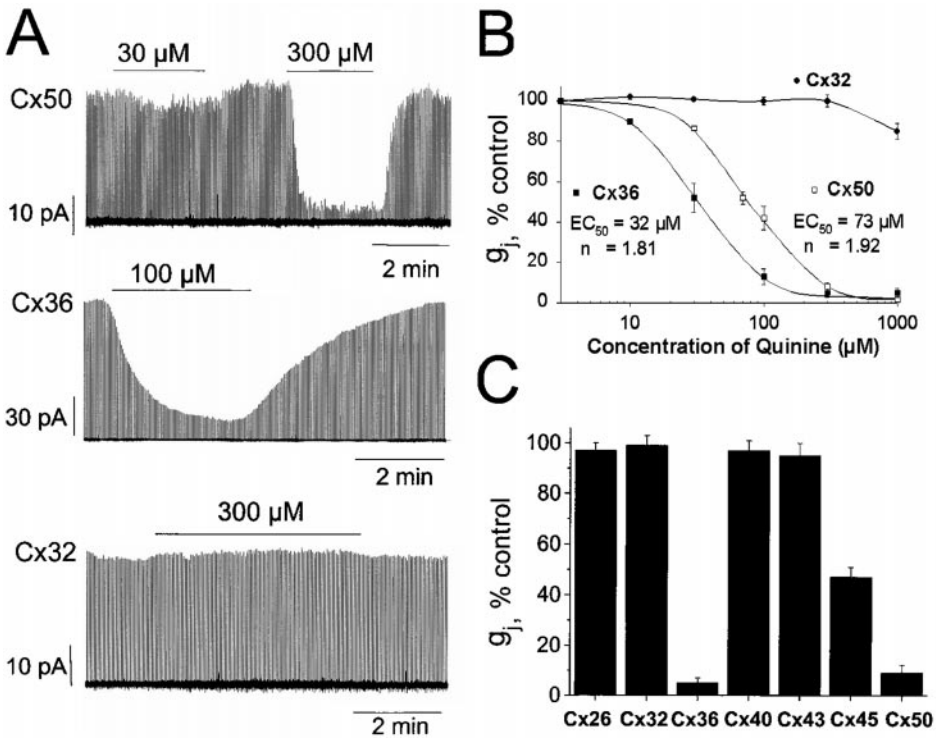

Fig. 1. Quinine reduces junctional conductance in a reversible, concentration-dependent, and connexin-selective manner. (*A*) Effect of indicated concentrations of quinine on junctional currents in response to 200-ms pulses to −10 mV applied to one cell of a pair every 2 s from a holding potential of 0 mV. Application of indicated concentrations of quinine decreased I_j of Cx50 (*Top*) and Cx36 channels (*Middle*) but not Cx32 channels (*Bottom*). (*B*) Concentration dependence of the effect of quinine on Cx36 (■), Cx50 (□), and Cx32 (●) gap junction channels. Each point represents the mean ± SEM of g_j (% of the initial conductance) values obtained from 4–18 cell pairs. The solid line is a fit of the data points to the Hill equation (see *Methods*). The EC_{50} and Hill slope values are indicated. (*C*) Bar graph illustrating that quinine (300 μM) has no significant effect on Cx26, Cx32, Cx40, and Cx43 gap junction channels. However, this concentration of quinine significantly inhibited Cx36, Cx45, and Cx50 channels. Each bar represents the mean ± SEM of 4–6 cell pairs.

of a pair. Gating events were recognized as simultaneously occurring events of equal amplitude and opposite polarity in current traces for both cells in the pair.

Quinine and *N*-benzylquininium (BQ) were obtained from Sigma and were dissolved in the extracellular or intracellular solutions to make 10 mM stock solution. Stock solutions were prepared daily. pH values of external and internal solutions were measured and adjusted before each experiment. Drugs were applied with a gravity-fed perfusion system. Solution exchanges were complete within 30 s. Concentration–response curves for quinine-induced block were determined by exposure of each cell pair to two to three concentrations of quinine. In all experiments, reversibility was assessed by washout of the drug. The magnitude of inhibition caused by quinine is expressed as the fraction of the conductance in the absence and presence of the drug, g_j, % control. Concentrations of quinine (Q) that caused a half-maximal inhibition (EC_{50}) and the Hill coefficients (n) of concentration–response relationships were estimated by fitting the data to the following equation:

$$g_j, \% \text{ control} = 1/[1 + ([Q]/EC_{50})^n].$$

Data are presented as means ± SEM.

Results

The effects of quinine on gap-junctional conductance of channels formed by several connexins are illustrated in Fig. 1. Fig. 1*A* shows the effect of external application of quinine on Cx50, Cx36, and Cx32 gap-junction channel currents (I_j). In the case of Cx50 channels, low concentrations of quinine (30 μM) decreased I_j by ≈10%, whereas the higher illustrated drug concentration (300 μM) caused near-maximal decrease in I_j. For both quinine concentrations, washout of the drug resulted in complete recovery of the currents; i.e., the block was reversible (Fig. 1*A Top*). In the case of Cx36 channels (Fig. 1*A Middle*), quinine (100 μM) decreased I_j by ≈86%, which was similarly reversible on washout. However, in the case of Cx36, the time course of recovery was significantly slower than that of Cx50 channels. For Cx32 gap junctions, exposure to 300 μM quinine was ineffective in reducing junctional currents (Fig. 1*A Bottom*).

Fig. 1*B* shows the concentration dependence of quinine-induced block of Cx36 and Cx50 junctional currents determined by exposure of 4–10 cell pairs to each drug concentration. Nonlinear least-squares fit of the individual data points to the Hill equation (see *Methods*) yielded EC_{50} values of 32 μM and 73 μM for the quinine-induced inhibition of Cx36 and Cx50 gap junction channels, respectively. In both cases, Hill coefficients were ≈2 (1.81 for Cx36, 1.92 for Cx50), indicating that binding of two molecules of quinine was required to block gap junction channels. The EC_{50} values for the effect of quinine are comparable to those found for block of potassium (22, 23) and volume-activated chloride channels (24) and are within the range of plasma concentrations achieved during antimalarial therapy (18–71 μM: ref. 25). Cx36 is highly expressed in the retina (26), pancreatic islet cells (27), and the brain (28), whereas Cx50 is expressed in the lens (29); thus, some of the side effects caused by quinine administration, such as vision loss and hypoglycemia (25), might result in part from gap junction blockade.

The block of gap junction channels depended on the connexin subtype. Fig. 1*A* shows that a high concentration of quinine, sufficient to cause near-maximal decreases in Cx36 and Cx50 channels, did not cause a detectable reduction of Cx32 junctional currents (varying by 2 ± 1%; $n = 6$). We tested the effects of quinine on gap junction channels formed by several other connexins, including Cx43, Cx45, Cx40 (the three major mammalian cardiac connexins), Cx35, and Cx34.7 (two distinct skate homologues of Cx36; ref. 30), and Cx26. As illustrated in Fig. 1*C*, quinine (300 μM) did not significantly inhibit Cx26, Cx32, Cx40, or Cx43 gap junction channel currents. Quinine-induced reduction of junction conductance was less than 10% in each of these cases (means ± SEM are 3 ± 1%, $n = 6$ for Cx26; 4 ± 1%, $n = 5$ for Cx40; and 8 ± 2%, $n = 7$ for Cx43). Higher concentrations of quinine (1 mM) increased the magnitude of inhibition of the current in the case of Cx32 and Cx43 channels (means ± SEM are 12 ± 4%, $n = 6$ for Cx32; and 16 ± 2%, $n = 4$ for Cx43). In the case of Cx45, 300 μM quinine induced a significant reduction

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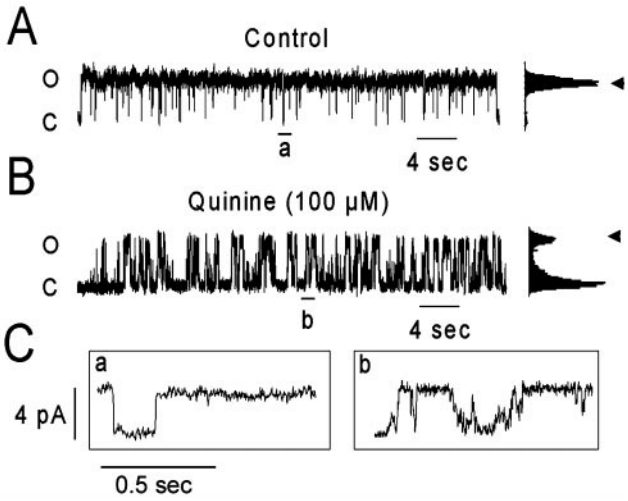

Fig. 2. Effect of quinine on single Cx50 channels at a V_j of −20 mV. (*A*) A 50-sec recording of Cx50 channels in the absence of quinine. Cx50 channels are predominantly open (O) at this V_j and exhibit rapid transitions as shown in the 1-sec segment of the recording (*C Inset a*). (*B*) Application of the quinine (100 μM) to the same cell pair caused a reduction in the open probability of the channels. Amplitude histograms shown to the right of each trace indicate that the conductance of the open state was not affected in the presence of 100 μM quinine. Arrowheads in both histograms indicate the peaks corresponding to the open state of 4.1 pA (in control) and 3.95 pA (in quinine). In the presence of quinine, the channel primarily exhibits ''slow'' transitions between the open and closed state (*C*), as indicated in the 1-sec segment of the recording in the presence of quinine (*Inset b*).

in the junctional current (n = 10). Quinine also significantly blocked gap junction channels formed by Cx35 and Cx34.7, fish homologues of Cx36 (ref. 29; data not shown); the magnitude of block induced by 300 μM quinine ranged from 60% to 83% for Cx35 (n = 3) and 75% to 88% for Cx34.7 (n = 3). These results demonstrate that the quinine-induced inhibition was restricted to Cx50, Cx45, and Cx36 and its fish homologues, and indicate that quinine selectively blocks gap junction channels.

Previous studies indicated that quinine (100 μM) does not block Cx35 gap junction channels when expressed in oocytes (20). Part of the discrepancy may arise because of the differences in the expression system. Differences in the potency of quinidine at blocking potassium channels when expressed in oocytes or mammalian cells have been observed (22, 23). Nevertheless, to determine whether the action of quinine was cell-type specific, we evaluated the effects of the drug on Cx36 and Cx43 channels when expressed in RIN cells. The inhibition by quinine (300 μM) of junctional conductance of Cx36 and Cx43 channels in RIN cells was similar to that found in N2A cells (values are 93 ± 5%, n = 5 for Cx36; and 3 ± 2%, n = 4 for Cx43). The similar efficacy of quinine on Cx36 and Cx43 channels expressed in two different cell lines strongly suggests that the action is connexin- rather than cell-type-specific.

Single-channel studies were undertaken to investigate the mechanism of channel closure by quinine on susceptible gap junction channels. Because the unitary conductance of Cx36 channels is extremely low (10–15 pS), the effect of quinine on single-channel gating was investigated primarily on Cx50 gap junctions. The effect of quinine (100 μM) on Cx50 single channels at a V_j of 20 mV applied to one cell of a pair is shown in Fig. 2. At this voltage, Cx50 channels are open >90% of the time in the absence of quinine (Fig. 2*A*; ref. 31). External application of quinine caused a decrease in the open probability (P_o) of the channel without appreciably modifying the single-channel conductance. The P_o of single Cx50 channels at 100 μM was 0.48, very close to the 58 ± 6% inhibition of g_j by this quinine concentration obtained in macroscopic measurements (Fig. 1*B*). Similarly, the P_o of single Cx50 channels at 30 and 300 μM quinine were 0.87 and 0.06, values that are close to the inhibition of g_j obtained from macroscopic currents. The single-channel conductances measured for Cx50 channels in the absence and presence of quinine were 209 ± 14 pS (n = 3), and 198 ± 13 pS (n = 3), respectively, which are not significantly different.

Quinine markedly modified the gating characteristics of single gap junction channels. In the absence of quinine, Cx50 channels exhibit fast transitions as described (ref 31; Fig. 2*C Inset a* for a 1-sec segment of the control trace). In contrast, gating of channels in the presence of quinine is primarily (greater than 90%) characterized by slow transitions between the open and fully closed state, as shown in the 1-sec segment of the recording in the presence of 100 μM quinine (Fig. 2*C Inset b*). Rise and/or decay times of these slow transitions varied between 8 and 200 msec. These slow transitions between the open and closed states were the predominant form of gating observed in the presence of quinine and were detected at other quinine concentrations (data not shown). The frequency of slow transitions was maximal at intermediate concentrations of quinine near the IC_{50} value.

Location of the Binding Site. The approach used here to determine the location of the binding site for quinine (a tertiary amine; pKa ≈ 8.7) is based on previous strategies used with sodium channel blockers such as local anesthetic molecules (32–35). Local anesthetics such as lidocaine are tertiary amines with pKa values in the range of 7 to 10, and thus at physiological pH can exist in both the uncharged free amine form and the cationic protonated form. Externally applied drugs cross the membrane in their uncharged form and bind to the receptor in their charged form after protonation in the aqueous environment of the cytoplasm. Thus, raising the external pH (pH_e), which increases the concentration of the uncharged form, increases the block rate and potency of tertiary amine drugs (32, 33), whereas increasing the internal pH (pH_i), which reduces the cationic form of the drug in the cytoplasm, has an opposite effect (34). Additional studies using permanently charged, lipid-insoluble quaternary derivatives of lidocaine further demonstrated that the active form of the drug is the charged form (34, 35).

We have similarly investigated the effect of external quinine at different pH_e (Fig. 3) and pH_i (Fig. 4) values and that of a quaternary derivative of quinine (Fig. 5). Fig. 3*A* shows the effect of 90-s exposure to quinine (100 μM) on Cx50 junctional currents at pH_e values of 7.0, 7.4, and 8.2 (where uncharged quinine proportions are calculated as 2%, 5%, and 32% by using the Henderson–Hasselbalch equation). Junctional currents were measured initially and during quinine washout in external solution at pH 7.4. Varying pH_e for 90 sec in the absence of quinine caused no significant change in the junctional conductance (see Fig. 3*B*, solid bars). As illustrated in Fig. 3*A*, the magnitude of block of Cx50 junctional currents by external quinine (100 μM) is markedly increased at high pH_e. Summaries of these experiments are illustrated in Fig. 3*B*. The magnitude of block induced by 100 μM quinine was 14 ± 3% at pH 7.0 (n = 4), 58 ± 6% at pH 7.4 (n = 17) and 91 ± 7% at pH 8.2 (n = 8). Similar results were obtained for Cx36 channels (data not shown).

High pH_i markedly reduced the potency of quinine. The effect of external application of 100 μM and 300 μM quinine on Cx50 gap junction channel currents when patch pipettes contained internal solutions at pH_i of 7.7 or 8.0 is illustrated in Fig. 4*A*. The time course of Cx50 junctional conductance decreases caused by 100 μM and 300 μM at three different pH_i values (i.e., 7.2, 7.7, and 8.0) are shown. The external pH was 7.4. At pH_i of 7.2 (Fig. 4*A Top*; also see Fig. 1*B*), 100 μM and 300 μM decreased Cx50 junctional currents by 58 ± 6% and 89 ± 3%, respectively. In contrast, when patch pipettes contained internal solutions at pH_i

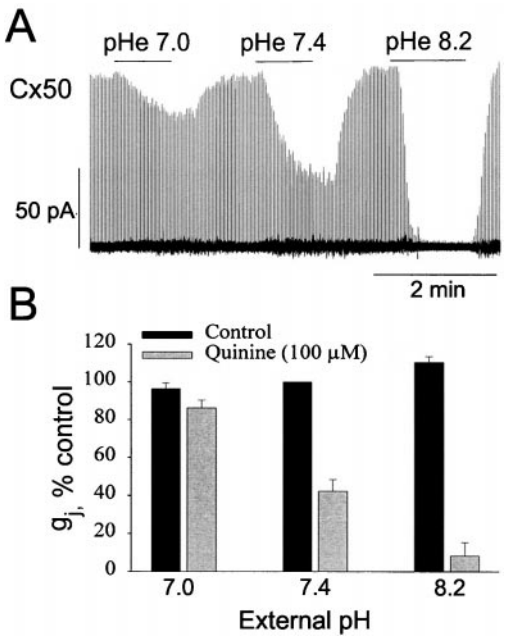

Fig. 3. pH_e affects the potency of quinine. *A*. The magnitude of block of Cx50 gap junction channels by 100 μM quinine (pKa $\approx$ 8.7) at pH_e values of 7.0, 7.4, and 8.2 is illustrated. The magnitude of inhibition by quinine is markedly increased at high pH_e. The pH of the pipette solution was 7.2. (*B*) Summary of experiments illustrated in *A*, showing the effect of 100 μM quinine at various pH_e values. Mean $\pm$ SEM of g_j (% of the initial conductance at pH 7.4) values in the presence of quinine (gray bars) at pH_e values of 7.0, 7.4, and 8.2 are shown. A 3-min exposure to solutions at pH_e values of 7.0 and 8.2 in the absence of quinine did not cause a significant change in g_j (% control at pH 7.4, solid bars).

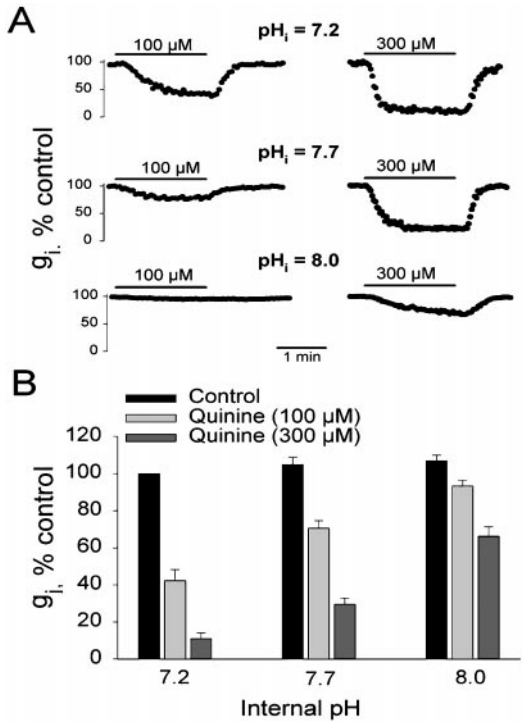

Fig. 4. pH_i affects the potency of quinine. (*A*) The time course of the effect of 100 μM and 300 μM quinine on Cx50 gap junction channel currents is illustrated for three pH values of the pipette solution, 7.2, 7.7, and 8.0. Each point represents the peak junctional conductance measured in response to 200-msec pulses to -10 mV applied to one cell of a pair every 2 sec from a holding potential of 0 mV. The effect of quinine at each pH_i value was measured in different cells at a pH_e value of 7.4. The magnitude of inhibition caused by both 100 and 300 μM quinine was markedly reduced at high pH_i values. (*B*) Bar graph illustrating the effect of 100 (light gray bars) and 300 (dark gray bars) μM quinine at pH_i values of 7.2, 7.7, and 8.0. The effect of pH_i values of 7.2, 7.7, and 8.0 in the absence of quinine is also illustrated (solid bars, control). Each bar represents mean $\pm$ SEM of g_j (% of the initial conductance) values measured in separate cell pairs in the absence and presence of quinine.

of 7.7 or 8.0, the magnitude of block caused by these concentrations was markedly reduced. The magnitudes of block caused by 100 μM and 300 μM quinine were 29 $\pm$ 4% and 71 $\pm$ 4% at pH_i 7.7 and 7 $\pm$ 3% and 37 $\pm$ 4% at pH_i 8.0 (Fig. 4*B*). These results clearly indicate that the potency of quinine at blocking Cx50 channels was reduced at high pH_i. The magnitude of block of Cx36 channels by quinine (30 μM) was also reduced at high pH_i (data not shown).

The reduction of the potency of quinine at high pH_i strongly suggests that the binding site is located intracellularly where extracellular quinine can gain access in its uncharged form and block in its charged form. However, alternative explanations might also account for the observed decrease in potency. For example, high pH_i might affect the binding site located in a hydrophobic region and thereby reduce the affinity of the drug or pH_i might affect steps distal to the binding step (i.e., the conformational change involved in channel closure). To provide further evidence for the existence of an intracellular binding site, we quantified the effects of a quaternary quinine derivative. Because such derivatives are permanently charged, they should be effective when applied internally, but would not be expected to gain access to the binding site when applied externally (see refs. 34 and 35).

The simplest quaternary derivative would be a methyl substituent on the tertiary nitrogen of quinine. However, such a derivative is not commercially available and therefore we used a quaternary benzyl derivative of quinine, BQ (425 Da). Results of these studies on Cx50 channels are illustrated in Fig. 5. External application of BQ (1 mM) did not cause a significant decrease in the junctional current of Cx50-expressing cells (mean = 4 $\pm$ 3%, n = 3). Exposure of these cells to 3 mM BQ for 4 min caused a slight decrease in the current that was reversible on washout (19 $\pm$ 4%, n = 4), which might be caused by slight membrane permeability of this polar molecule or to an additional low-affinity binding site. In contrast, addition of BQ to the patch pipettes caused a time-dependent reduction in I_j. The decrease in I_j was 98 $\pm$ 2% at 3 mM BQ (n = 4) and 97 $\pm$ 3% (n = 6) at 1 mM BQ. At a lower concentration (300 μM), BQ decreased the current by 71 $\pm$ 4.6% (n = 4) within 14 min of achieving the whole-cell configuration. The magnitude of inhibition of internally applied BQ was not appreciably altered when patch pipettes contained solutions at pH_i of 8.0 (data not shown). Single-channel studies indicate that BQ elicits slow transitions much like those observed with quinine (Fig. 5*C*).

The effect of internal BQ on other quinine-sensitive and quinine-insensitive connexins was also determined. Cx26 and Cx43 channels, which were not blocked by quinine, were similarly insensitive to BQ (data not shown). Cx45 channels, which were weakly sensitive to quinine, were only partially blocked by BQ and only at very high concentrations (data not shown; 48 $\pm$ 8%; n = 4 at 10 mM BQ). However, in the case of Cx36 and its fish homologue Cx35, internal application of even high concentrations of BQ (10 mM) did not cause significant blockade of the current (n = 8 for Cx36). The implication for the lack of effect of BQ is discussed below.

Discussion

Our results indicate that quinine closes gap junction channels in a reversible, concentration-dependent and connexin-specific

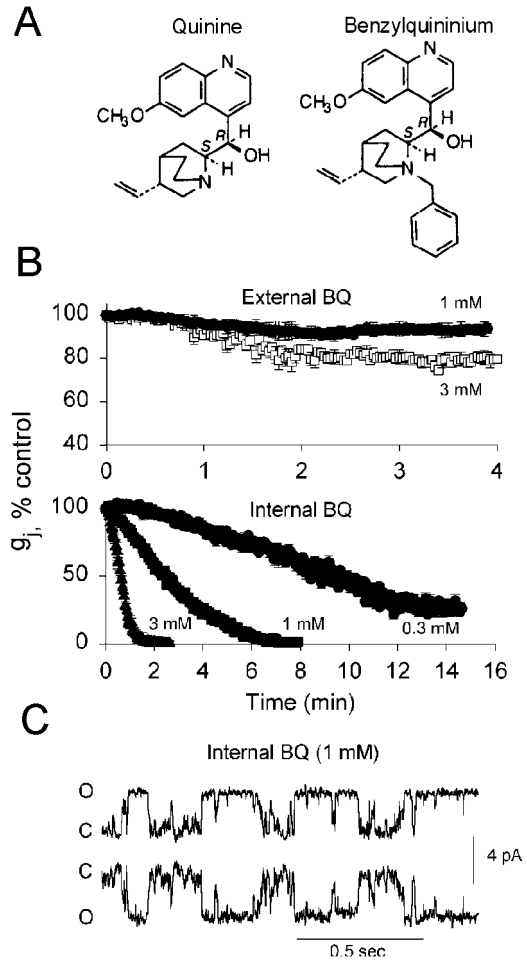

Fig. 5. The effect of external and internal application of indicated concentrations of a quaternary derivative of quinine, BQ, on Cx50 channel conductance. (*A*) Structures of quinine and BQ. (*B Upper*) External application of 1 mM BQ did not cause a significant decrease in the junctional current (■). Exposure of cells to 3 mM BQ for 4 min caused a slight decrease in the current (□). (*Lower*) Applied internally by addition to the patch pipettes, BQ at 0.3 (●), 1 (■), and 3 (▲) mM caused a marked decrease in the channel conductance in a time-dependent manner. (*C*) Single-channel events during closure of Cx50 channels by BQ (1 mM) at a V_j of −20 mV. Slow gating characteristics in the presence of BQ are similar to those observed in the presence of quinine. Simultaneously occurring events of equal amplitude and opposite polarity in current traces for both cells in the pair are shown. The upper trace represents the junctional current.

manner. Cx36 and Cx50 are most potently blocked by quinine, whereas other connexins were only weakly blocked by the drug. The location of the binding site of quinine was investigated by determining the effect of quinine at a range of intracellular and extracellular pH values. For Cx50 channels, access for quinine from the extracellular solution to its intracellular receptor site occurs initially through a hydrophobic pathway, as suggested by experiments illustrated in Fig. 3, which showed that increasing the external concentration of the uncharged form markedly increased the magnitude of block. The uncharged drug crosses the membrane and may cause blockade by binding to the receptor, in either its uncharged or its protonated form. Our experiments are strongly indicative of the latter possibility. That the intracellular concentration of the charged form of the drug is the major determinant of block is indicated by experiments at high pH_i (Fig. 4), which showed that reducing the concentration of this form markedly reduces the potency of quinine. Additional evidence for an intracellular binding site was provided by experiments with the quaternary derivative, which produced significant blockade of the Cx50 channels when applied internally but not when added externally (Fig. 5). The experiments with BQ further indicate that the site in Cx50 channels is accessible to the drug when introduced into the cytoplasm. Thus, for Cx50 channels, our results clearly indicate that the site is intracellular, possibly within the pore.

In the case of Cx36 channels, the location of the binding site for quinine remains to be conclusively determined. Although preliminary experiments indicated that the potency of quinine block of Cx36 channels is modulated by external and internal pH in the same way as Cx50 channels (data not shown), suggesting an intracellular binding site, the lack of an effect of the quaternary derivative makes the proposal less definitive. One reason for the lack of internally applied BQ may be the limited access of the drug to its binding site when introduced into the cytoplasm. Dye-coupling studies indicate that positively charged dyes such as 4′,6-diamidino-2-phenylindole (360 Da) do not permeate Cx36 channels (14), and that it is possible that the access of positively charged BQ to the receptor site is limited by regions that influence permeation. Nevertheless, conclusive evidence for an intracellular binding site awaits additional studies.

Single-channel studies on Cx50 channels are suggestive of an effect of quinine on a gating process, although other mechanisms including open channel block cannot be conclusively ruled out. Slow gating transitions of the sort illustrated in Fig. 2 have been observed previously in hemichannels at hyperpolarized membrane potentials, where they have been attributed to a ''loop'' gate, and in response to a multitude of uncoupling agents including low pH, heptanol, halothane, and carbenoxolone, where they have been attributed to a ''chemical'' gate (refs. 8, 11, and 37–40 and our own unpublished observations). Thus, the slow transitions observed in the presence of quinine may reflect its action on the ''chemical'' or the ''loop'' gate. An effect through gating rather than open channel block is also consistent with lack of blockade of Cx35 hemichannels by quinine in oocytes (although the action of quinine on hemichannels and gap junction channels may involve distinct mechanisms). Nevertheless, additional studies determining the effect of voltage and permeant ions on quinine-induced block are clearly necessary to clarify the mechanism of action of quinine.

Conclusions

The lack of naturally occurring gap junction channel blockers has, in part, been attributed to the inaccessibility of gap junctions to extracellular space and the large pore size of these channels. However, our results indicate that it is possible for a small, naturally occurring tertiary-amine compound (360 Da) to have significant effects on gap junction channels at EC_{50} values comparable to its action on other ion channels. In addition, this compound pharmacologically discriminates between various connexin subtypes, a hitherto undemonstrated concept. Although the development of compounds that selectively modify certain gap junction channels would be expected to be useful for delineating the roles of specific connexin subtypes (as might be expected for quinine blockade of neuronal gap junctions), a major drawback is that quinine is not specific for gap junction channels. Structure–activity studies of the quinine-based antimalarial drugs will perhaps lead to the synthesis of a quinine-based derivative that will block gap junction channels in a specific manner.

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