Effects of Quinine, Quinidine, and Chloroquine on α9α10 Nicotinic Cholinergic Receptors

Jimena A. Ballesteros, Paola V. Plazas, Sebastian Kracun, María E. Gómez-Casati, Julián Taranda, Carla V. Rothlin, Eleonora Katz, Neil S. Millar, and A. Belén Elgoyhen

Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Consejo Nacional de Investigaciones Científicas y Técnicas (J.A.B., P.V.P., M.E.G.-C., J.T., C.V.R., E.K., A.B.E.), and Departamento de Fisiología, Biología Molecular y Celular, FCEyN, Universidad de Buenos Aires, Buenos Aires, Argentina (E.K.); and Department of Pharmacology, University College London, London, United Kingdom (S.K., N.S.M.)

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ABSTRACT

In this study, we report the effects of the quinoline derivatives quinine, its optical isomer quinidine, and chloroquine on α9α10-containing nicotinic acetylcholine receptors (nAChRs). The compounds blocked acetylcholine (ACh)-evoked responses in α9α10-injected Xenopus laevis oocytes in a concentration-dependent manner, with a rank order of potency of chloroquine (IC50 = 0.39 μM) > quinine (IC50 = 0.97 μM) > quinidine (IC50 = 1.37 μM). Moreover, chloroquine blocked ACh-evoked responses on rat cochlear inner hair cells with an IC50 value of 0.13 μM, which is within the same range as that observed for recombinant receptors. Block by chloroquine was purely competitive, whereas quinine inhibited ACh currents in a mixed competitive and noncompetitive manner. The competitive nature of the blockage produced by the three compounds was confirmed by equilibrium binding experiments using [3H]methyllycaconitine. Binding affinities (Kv values) were 2.3, 5.5, and 13.0 μM for chloroquine, quinine, and quinidine, respectively. Block by quinine was found to be only slightly voltage-dependent, thus precluding open-channel block as the main mechanism of interaction of quinine with α9α10 nAChRs. The present results add to the pharmacological characterization of α9α10-containing nicotinic receptors and indicate that the efferent olivocochlear system that innervates the cochlear hair cells is a target of these ototoxic antimalarial compounds.

Quinoline derivatives such as quinine, quinidine, and chloroquine are well known for their use in the treatment of malaria. Their side effects on the auditory system have long been recognized and include reversible (but sometimes permanent) sensorineural hearing loss, tinnitus, and vertigo (Jung et al., 1993). The mechanism of ototoxicity may involve different levels of the auditory system (Eggermont and Kenmochi, 1998; Jarboe and Hallworth, 1999). However, there is considerable evidence showing that the auditory periphery is the primary location underlying the reversible hearing loss induced by quinine (Puel et al., 1990; Lin et al., 1998). Perfusion of quinine into the perilymphatic space of guinea pig cochlea can result in a reduction of the compound action potential, cochlear microphonic, and summing potential (Puel et al., 1990). In addition, it can affect the electromotility of outer hair cells (Zheng et al., 2001). Moreover, it can inhibit the K+ current of outer hair cells and both the K+ and Na+ currents of the spiral ganglion cells (Lin et al., 1998), thus indicating a variety of effects of this compound on different ion channels.

It has been reported that quinine and quinidine can also block acetylcholine (ACh)-induced K+ currents in outer hair cells and influence the effect of ACh on the compound action potential, suggesting a putative effect on the olivocochlear efferent system physiology (Daigneault et al., 1970; Yamamoto et al., 1997). Pharmacological and biophysical studies performed with the native cholinergic receptors present in mammalian and chicken hair cells (Fuchs, 1996) and cellular localization data (Elgoyhen et al., 1994, 2001; Lustig et al., 2001; Sgard et al., 2002) strongly suggest that the native receptor present at the efferent cholinergic olivocochlear-outer and developing inner hair cell synapse is as-

ABBREVIATIONS: ACh, acetylcholine; nAChR, nicotinic acetylcholine receptors; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid; I-V, current-voltage relationship; MLA, methyllycaconitine; nAChR, nicotinic acetylcholine receptor; 5-HT, 5-hydroxytryptamine.
sembled from both the α9 and α10 nicotinic subunits (Elgoyhen et al., 1994, 2001). Thus, α9α10-containing nicotinic acetylcholine receptors (nAChRs) might be targets of the effects of quinoline compounds within the auditory system. In this regard, aminoglycosides, ototoxic drugs not related in structure to the quinoline compounds, have been reported as blockers of the α9α10 nAChRs (Rothlin et al., 2001), pointing this receptor as a possible site of interaction of ototoxic drugs. Moreover, the interaction of quinine concentrations greater than 50 μM with nAChRs has been reported for receptors present at the neuromuscular junction, in which it produces long-lived open-channel as well as a closed-channel block and can normalize the open duration of channel events in the slow-channel congenital myasthenic syndrome (Sieb et al., 1996; Fukudome et al., 1998).

We have examined the effects of the quinoline derivatives quinine, quinidine, and chloroquine (Fig. 1) on recombinant α9α10 nAChRs, reconstituted in Xenopus laevis oocytes. We show evidence that these compounds block the α9α10 nAChRs. The underlying mechanisms range from competitive in the case of chloroquine to mixed competitive and noncompetitive in the case of quinine. Moreover, we demonstrate that chloroquine blocks the native α9α10-containing nAChRs of inner hair cells. The present results indicate that the efferent olivocochlear system that innervates the cochlear hair cells is a direct target of these ototoxic antimalarial compounds.

Materials and Methods

Expression of Recombinant Receptors in X. laevis Oocytes. For expression studies, α9 and α10 rat nAChR subunits were subcloned into a modified pGEMHE vector (Liman et al., 1992). Capped cRNAs were in vitro-transcribed from linearized plasmid DNA templates using the mMessage mMachine T7 Transcription Kit (Ambion, Austin, TX). The maintenance of X. laevis and the preparation and cRNA injection of stage V and VI oocytes have been described in detail elsewhere (Katz et al., 2000). Oocytes were typically injected with 50 nl of RNase-free water containing 0.01 to 1.0 ng of cRNAs (at a 1:1 M ratio) and maintained in Barth's solution at 17°C.

Electrophysiological recordings were performed 2 to 6 days after cRNA injection under two-electrode voltage-clamp with a Geneclamp 500 amplifier (Axon Instruments Inc., Union City, CA). Both voltage and current electrodes were filled with 3 M KCl and had resistances of ~1 to 2 MΩ. Data acquisition was performed using a Digidata 1200 and the pClamp 7.0 software (Axon Instruments). Data were analyzed using ClampFit from the pClamp 6.1 software. During electrophysiological recordings, oocytes were continuously superfused (~10 ml/min) with normal frog saline composed of 115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl2, and 10 mM HEPES buffer, pH 7.2. Unless otherwise indicated, the membrane potential was clamped to −70 mV. Drugs were applied in the perfusion solution of the oocyte chamber. To minimize activation of the endogenous Ca2+ -sensitive chloride current (Elgoyhen et al., 2001), all experiments were performed in oocytes incubated with the Ca2+ chelator BAPTA-ace-toxymethyl ester (100 μM) for 3 to 4 h before electrophysiological recordings.

Concentration-response curves were normalized to the maximal agonist response in each oocyte. For the inhibition curves, antagonists were added to the perfusion solution for 2 min before the addition of 10 μM ACh and then were coapplied with this agonist. Responses were referred to as a percentage of the response to ACh. The mean and S.E.M. values for peak current responses are represented. Agonist concentration-response curves were iteratively fitted with the equation

\[ I_{\text{max}} = A / (A^* + EC_{50}^*) \]

where \( I \) is the peak inward current evoked by agonist at concentration \( A \), \( I_{\text{max}} \) is the current evoked by the concentration of agonist eliciting a maximal response, \( EC_{50}^* \) is the concentration of agonist inducing half-maximal current response, and \( n \) is the Hill coefficient. An equation of the same form was used to analyze the concentration-dependence of antagonist-induced blockage. The parameters derived were the concentration of antagonist producing a 50% block of the control response to ACh (IC50) and the associated interaction coefficient (n). Analysis of competitive inhibition was performed by the Schild plot (Arunlakshana and Schild, 1959), with the following equation:

\[ \log[(A'/A) - 1] = \log B - \log K_B \]

where \( A \) and \( A' \) are the EC50 values of ACh in the absence and presence of antagonist, respectively; \( B \) is the concentration of antagonist, and \( K_B \) is the equilibrium dissociation constant for the combination of the antagonist with the receptor. Further analysis was performed using the Gaddum-Schild equation as recommended (Neubig et al., 2003):

\[ pEC_{50} = -\log(B - 10^{-pA2}) - \log c \]

where \( pEC_{50} \) is the negative logarithm of the EC50 of the agonist, \( pA2 \) is the negative logarithm of the molar concentration of the antagonist that makes it necessary to double the concentration of the agonist needed to elicit the original response obtained in the absence of antagonist, \( B \) is the antagonist concentration, \( S \) is the logistic slope factor, and \( \log c \) is a fitting constant. When \( S \) equals 1, \( pA2 \) corresponds to the negative logarithm of \( K_B \). The analysis of the correlation of IC50 values as a function of the concentration of agonist was performed by the Cheng-Prusoff equation (Cheng and Prusoff, 1973), with the modification introduced by Leff and Dougall (1993):

\[ IC_{50} = K_B[2 + (A/EC_{50})^{n+1}] - 1 \]

where \( n \) is the Hill coefficient, \( A \) is the agonist concentration, and the other parameters are as defined above.

Current-voltage (I-V) relationships were obtained by applying 2-s voltage ramps from +50 to −120 mV 5 s after the peak response to ACh from a holding potential of −70 mV. Leakage correction was performed by digital subtraction of the I-V curve obtained by the same voltage-ramp protocol before the application of ACh. Generation of voltage protocols and data acquisition were performed using a Digidata 1200 and the pClamp 6.1 or 7.0 software (Axon Instruments). Data were analyzed using ClampFit from the pClamp 6.1 software.

Electrophysiological Recordings from Hair Cells. Apical turns of the organ of Corti were excised from Sprague-Dawley rats at postnatal ages P9–P11 and were used within 3 h. The day of birth was considered postnatal day 0 (P0). Cochlear preparations were...
mounted under an Axioscope microscope (Carl Zeiss GmbH, Oberkochem, Germany) and were viewed with differential interference contrast using a 63× water-immersion objective and a camera with contrast enhancement (Hamamatsu C2400-07; Hamamatsu Corporation, Hamamatsu City, Japan). Methods of recording from inner hair cells were essentially as described previously (Katz et al., 2004).

In brief, inner hair cells were identified visually with the 63× objective lens and during recordings by the size of their capacitive (7 to 12 pF), by their characteristic voltage-dependent Na+ and K+ currents, and at older ages, including a fast-activating K+ conductance (Kres et al., 1998). Some cells were removed to access the inner hair cells, but mostly, the pipette moved through the tissue under positive pressure. The extracellular solution was 155 mM NaCl, 5.8 mM KCl, 1.3 mM CaCl2, 0.9 mM MgCl2, 0.7 mM NaH2PO4, 5.6 mM d-glucose, and 10 mM HEPES buffer, pH 7.4. The pipette solution contained 150 mM KCl, 3.5 mM MgCl2, 0.1 mM CaCl2, 5 mM BAPTA buffer, and 2.5 mM Na2ATP, pH 7.2. To minimize even more the contribution of small-conductance Ca2+-activated K+ in addition to using BAPTA in the pipette solution, the K+ channel blocker APM (1 mM) was added to the external working solutions. Glass pipettes, 1.2-mm inside diameter had resistances of 7 to 10 MΩ. Experiments were done at a holding voltage of ~90 mV, and all working solutions were made up in a saline containing only 0.5 mM Ca2+ and no Mg2+ so as to optimize the experimental conditions for measuring currents flowing through the α9α10 receptors (Weisstaub et al., 2002; Katz et al., 2004). Solutions were applied by a gravity-fed multichannel glass pipette (~150 μm tip diameter) positioned approximately 300 μm from the recorded cell. Currents were recorded in the whole-cell patch-clamp mode with an Axopatch 200B amplifier, low-pass filtered at 2 to 10 kHz, and digitized at 5 to 20 kHz with a Digidata 1200 board (Axon Instruments). Recordings were made at room temperature (22–25°C). Holding potentials were not corrected for liquid junction potentials or for the voltage drop across the uncompensated series resistance.

Mammalian Cell Culture and Transfection. Mammalian cell line, tsA201, derived from the human embryonic kidney 293 cell line, were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen, Paisley, UK) containing 2 mM l-Glutamax (Invitrogen) plus 10% heat-inactivated fetal calf serum (Sigma Chemical, Poole, Dorset, UK) with penicillin (100 U/ml) and streptomycin (100 μg/ml) and were maintained in a humidified incubator containing 5% CO2 at 37°C. Cells were cotransfected with pR5-α91L209/75HT1A and pR5-α101L209/75HT1A (Baker et al., 2004) using Effectene transfection reagent (QIAGEN, Crawley, UK) according to the manufacturer’s instructions. Cells were transfected overnight and assayed for expression approximately 40 to 48 h after transfection.

Radioligand Binding. Binding studies with [3H]methyllycaconitine ([3H]MLA; Tocris Cookson Inc., Avonmouth, UK; specific activity, 26 Ci/mmol) to cell membrane preparations were performed, essentially as described previously (Baker and Millar, 2004). Membranes (typically 80–150 mg of protein) were incubated with radioligand for 150 min at 4°C in a total volume of 150 μl in the presence of protease inhibitors leupeptin (2 μg/ml) and pepstatin (1 μg/ml). Radioligand binding was assayed by filtration onto Whatman GF/B filters (presoaked in 0.5% polyethyleneimine), followed by rapid washing with cold 10 mM phosphate buffer using a Brandel cell harvester. Bound radioligand was quantified by scintillation counting. Curves for equilibrium binding were fitted with the Hill equation by equally weighted least-squares (CVFIT program; David Colquhoun, University College London, London, UK). IC50 values were converted to Ki values by applying the Cheng-Prusoff correction.

Statistical Analysis. Statistical significance was evaluated by the Student’s t test (two-tailed, unpaired samples). Multiple comparisons of IC50 values were performed with a one-way analysis of variance followed by Tukey’s test. A p value <0.05 was considered significant.

Materials. ACh chloride, quinine hemisulfate, quinidine chloride, and chloroquine diphosphate were purchased from Sigma Chemical Co. (St. Louis, MO). BAPTA-acetoxymethyl ester (Molecular Probes, Eugene, OR) was stored at −20°C as aliquots of a 100 mM solution in dimethyl sulfoxide, thawed, and diluted 1000-fold into saline solution shortly before incubation of the oocytes.

All experimental protocols were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory animals (National Institutes of Health Publications 80-23), revised in 1978.

Results

Effect of Antimalarial Quinoline Derivatives on the α9α10 Recombinant nAChR. Figure 2A shows representative responses to 10 μM ACh (i.e., a concentration close to the EC50) (Elgoyhen et al., 2001) of X. laevis oocytes injected with α9 and α10 cRNAs and block of these responses in the presence of either quinine, quinidine, and chloroquine at a membrane potential of ~70 mV. The amplitude of the ACh

Fig. 2. Effect of quinoline derivatives on ACh-evoked currents through recombinant α9α10 nAChRs. A, representative traces to ACh either alone or in the presence of quinine, quinidine, or chloroquine. B, inhibition curves performed by the coapplication of 10 μM ACh and increasing concentrations of the compounds. Oocytes were incubated with α9 and α10 cRNAs and block of these responses in the presence of either quinine, quinidine, and chloroquine at a membrane potential of ~70 mV. The amplitude of the ACh...
responses was markedly reduced at micromolar concentrations of the quinoline derivatives. To evaluate the potency of the compounds, inhibition curves were carried out (Fig. 2B). Currents evoked by ACh were blocked in a concentration-dependent manner with a rank order of potency of chloroquine (IC_{50} = 0.39 ± 0.08 μM, Hill coefficient = 1.2 ± 0.1, n = 6) > quinine (IC_{50} = 0.97 ± 0.07 μM, Hill coefficient = 1.37 ± 0.29, n = 6) > quinidine (IC_{50} = 1.28 ± 0.05 μM, Hill coefficient = 1.2 ± 0.1, n = 6), p < 0.05. Block by antagonists was reversible because initial control responses to ACh were recovered after washes of the oocytes with frog saline for 3 min. Moreover, antagonists did not elicit per se responses in oocytes expressing α9α10 receptors.

**Underlying Mechanisms of Block.** The mechanism underlying the blocking action of chloroquine, quinine, and quinidine on the α9α10 receptor was further analyzed on recombinant receptors. Thus, concentration-response curves to ACh were performed in the absence or presence of the different antagonists (Fig. 3). At 1 μM, both quinine (Fig. 3A) and quinidine (Fig. 3B) shifted the concentration-response curves to ACh to higher concentrations without altering its maximal response, a behavior typical of a competitive antagonist. At higher concentrations of the antagonists, however, the maximal evoked ACh response was reduced (Fig. 3, A and B, and Table 1), indicating an additional effect. A shift to the right in the concentration-response curve, with concomitant insurmountable antagonistic effects at high concentrations of the agonist, is indicative of a noncompetitive inhibition. In contrast, chloroquine produced parallel rightward shifts in the ACh concentration-response curves, with increases in the EC_{50}. Moreover, it seemed to be a purely competitive antagonist, because full recovery of maximal ACh responses could be obtained even in the presence of a 10 μM concentration, which is 26-fold higher than its IC_{50} value (Fig. 3C and Table 2). Higher concentrations of chloroquine could not be tested, because the degree of block was so high that desensitizing

![Fig. 3. Mechanism of blockage of ACh-evoked responses by quinoline derivatives. A, concentration-response curves to ACh performed either alone (■) or in the presence of 1 (▲), 3 (▲), or 10 μM (▼) quinine. B, same experiment as in A but in the presence of quinidine. C, same as in A but in the presence of 0.3 (○), 1 (▲), 5 (●), or 10 μM (▼) chloroquine. Peak current values were normalized and referred to the maximal peak response to ACh. The mean and S.E.M. of 4 to 12 experiments per group are shown.](image)

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<tr>
<th>Quinine</th>
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<th>Quinidine</th>
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<tr>
<td>EC_{50}</td>
<td>Max</td>
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<tr>
<td>μM</td>
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<tr>
<td>0 μM</td>
<td>12.4 ± 0.3</td>
<td>0.9 ± 0.1</td>
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<tr>
<td>1 μM</td>
<td>41.8 ± 7.5</td>
<td>1.0 ± 0.1</td>
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<tr>
<td>3 μM</td>
<td>41.3 ± 9.1</td>
<td>0.6 ± 0.1</td>
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<tr>
<td>10 μM</td>
<td>71.4 ± 7.1</td>
<td>0.3 ± 0.1</td>
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H, Hill coefficient; n, number of experiments.

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<th>Chloroquine</th>
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<td>Concentration</td>
<td>EC_{50}</td>
<td>Max</td>
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<td>μM</td>
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<td>0 μM</td>
<td>13.2 ± 0.3</td>
<td>0.9 ± 0.1</td>
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<tr>
<td>0.3 μM</td>
<td>30.9 ± 2.1</td>
<td>0.9 ± 0.1</td>
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<tr>
<td>1 μM</td>
<td>42.2 ± 2.2</td>
<td>1 ± 0.1</td>
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<td>5 μM</td>
<td>171.5 ± 22.9</td>
<td>0.8 ± 0.1</td>
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<tr>
<td>10 μM</td>
<td>357.2 ± 44.9</td>
<td>0.9 ± 0.1</td>
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H, Hill coefficient; n, number of experiments.
materials and Methods). This equation predicts the IC₅₀ values introduced by Leff and Dougall (1993) (see eq. 4 under antagonists. The data from these experiments were then used and inhibition was observed at high concentrations of the antagonist. The theoretical curves were drawn using an EC₅₀ value for ACh of 13 μM, a Hill coefficient of 1.2 derived from ACh concentration-response curves (Fig. 3 and Elgoyhen et al., 2001), and a Kᵦ value of 0.73 μM for quinine (calculated using the Leff-Dougall eq. 4 for an IC₅₀ value of 0.97 μM, derived from Fig. 2B) and 0.39 μM for chloroquine (derived from data in Fig. 2B). As observed in Fig. 5A, in the case of quinine, the experimental IC₅₀ values adjusted to the theoretical curve at concentrations lower than 100 μM ACh, thus suggesting competitive inhibition. At higher concentrations of ACh, the experimental values deviated from those estimated by the equation, thus confirming additional noncompetitive block by quinine. On the other hand, in the case of chloroquine, all experimental data fitted the equation, thus confirming the competitive nature of this antagonist (Fig. 5B). Moreover, the same Kᵦ value as derived from the Schild analysis was obtained.

Because quinine is a positively charged molecule at physiological pH, an interaction with the pore of the channel could account for the noncompetitive type of block. If this were the case, then the blockage should be reduced at depolarized compared with hyperpolarized potentials. To evaluate the voltage-dependence of the quinine block, 2-s voltage ramp protocols (+50 to −120 mV) were performed in the presence of 300 μM ACh either alone or coapplied with 10 μM quinine. As shown in the representative I-V curves in Fig. 6A, blockage by quinine was more pronounced at hyperpolarized potentials. Figure 6B shows the current amplitude values derived from I-V curves like the one shown in Fig. 6A. Block by quinine was dependent on the membrane holding potential, being significantly larger at hyperpolarized than at depolarized holding potentials (Iₘₚ/ₚₖₖ = 0.13 ± 0.02 and 0.35 ± 0.05 at −120 and +50 mV, respectively; p < 0.05, n = 7). However, an e-fold difference in Iₘₚ/ₚₖₖ was only attained every
160 mV, thus indicating only a slight dependence on membrane potential.

**Competition Binding Data with Chloroquine, Quinidine, and Quinidine.** Competition radioligand binding was used to examine whether chloroquine, quinidine, and quinidine were able to displace binding of the high-affinity nicotinic antagonist methylycaconitine. Experiments were performed in transfected tsA201 cells using subunit chimeras (a9L209/5HT3A and a10L206/5HT3A) in which the extracellular N-terminal domain of the a9 or a10 subunits were fused to the transmembrane and intracellular domain of the mouse 5HT3A subunit (Baker et al., 2004). These chimeric receptors express very well in transfected cells (compared with wild-type a9a10 receptors), binding experiments give good specific-to-nonspecific signals, and chimeric receptors retain the same pharmacological profile compared with wild-type receptors expressed in X. laevis oocytes. As described previously, [3H]MLA binds with high affinity (Kd = 7.5 ± 1.2 nM) to cells coexpressing the a9 and a10 subunit chimeras (Baker et al., 2004). Equilibrium competition binding studies were performed with chloroquine, quinidine, and quinidine to determine their affinity for a9a10 receptors (Fig. 7). In all cases, complete displacement of bound [3H]MLA was observed, providing evidence of competitive binding to a single high-affinity site. Binding affinities (Ki values) determined from three independent experiments were the following: chloroquine, 2.3 ± 0.5 μM; quinidine, 5.5 ± 0.7 μM; and quinidine, 13.0 ± 2.9 μM. Thus, the rank order of potency was similar to that obtained from functional data in X. laevis oocytes.

**Chloroquine Blocks Native α9α10-Containing nAChRs of Rat Cochlear Inner Hair Cells.** It is currently accepted that olivocochlear efferent innervation to developing inner hair cells is subserved by an nAChR composed of both a9 and a10 nicotinic subunits (Fuchs, 1996; Elgoyhen et al., 2001; Lustig et al., 2001; Katz et al., 2004). We therefore studied the effects of chloroquine, the most potent blocking agent, on ACh responses (measured in isolation from the small-conductance Ca2+-activated K+ channels) in inner hair cells from acutely excised organs of Corti of P9–P11 rats as a source of native receptors. As shown on the representative traces of Fig. 8A and on the concentration-response curves of Fig. 8B, similar to those described for recombinant a9a10 receptors, chloroquine blocked responses to 60 μM ACh (the EC50 value in this system) (Katz et al., 2004) with an IC50 value of 0.13 ± 0.01 μM, n = 5.

**Discussion**

In the present study, we analyzed the effects of the quinoline compounds quinidine, its optical isomer quinidine, and chloroquine on the recombinant a9a10 nAChR expressed in X. laevis oocytes. We report on the basis of functional data that, although quinine and quinidine blocked ACh responses through a mixed competitive and noncompetitive mechanism, chloroquine blocked the receptors through a competitive mechanism. The competitive nature of the block produced by the three compounds was verified by ligand-binding experiments. Moreover, in the case of chloroquine, the blocking action was also observed on native a9a10-containing receptors of rat cochlear inner hair cells.

The observation that block by chloroquine was purely competitive indicates that this compound shares with ACh at least part of the binding pocket in such a way that occupancy of the site is mutually exclusive. ACh and all other agonists and competitive antagonists contain a positively charged quaternary ammonium group or a tertiary nitrogen group.

![Fig. 6. Block by quinine as a function of the membrane potential. A, representative I-V curves obtained upon application of 2-s voltage ramps (+50 to −120 mV), 5 s after the peak response to 300 μM ACh, either alone or in the presence of 10 μM quinidine. B, inhibition of responses to 300 μM ACh in the presence of 10 μM quinidine at different holding potentials. Current amplitudes in the presence of quinine plus ACh were obtained from I-V curves as shown in A and are expressed as the percentage of the control current amplitude with 300 μM ACh at each holding potential. The mean and S.E.M. of seven experiments per group are shown.](image1)

![Fig. 7. Competition radioligand binding to transfected mammalian cells. Cultured mammalian tsA201 cells were cotransfected with subunit chimeras a9L209/5HT3A and a10L206/5HT3A (in which the extracellular N-terminal domain of the a9 or a10 subunits are fused to the transmembrane and intracellular domain of the mouse 5HT3A subunit). Competition-binding data with chloroquine, quinidine, and quinidine is presented as a percentage of [3H]MLA binding obtained in the absence of competing ligand. The curves are from a single experiment but are typical of three independent experiments.](image2)
that can be protonated and that interacts with electron-rich side chains of aromatic residues within the nAChRs (Karlin, 2002). In this regard, chloroquine, quinine, and quinidine fulfill these criteria (Fig. 1). Although a structure-function study is beyond the scope of this work, the fact that quinine and quinidine produced an additional noncompetitive mechanism of block should derive from the fact that different from chloroquine, these compounds have a quinuclidine ring (Fig. 1) attached to the quinoline moiety (Tracy and Webster, 2001). This might generate an additional site of interaction with the receptor.

If the interaction of quinine with the α9α10 nAChR requires the entrance into the transmembrane field, then depolarization should reduce inhibition. The simplest explanation for a voltage-dependent block is that the blocking molecule either has a binding site within the channel, part-way across the electric field of the membrane (i.e., an open-channel blocker), or that it docks within the channel vestibule impairing ion flow. However, as derived from Fig. 6, an approximate e-fold difference in \( I/I_{\text{max}} \) was achieved every 160 mV. This indicates that blockage by quinine was only slightly dependent on the membrane potential and could imply that the blocker binds very near to the entrance of the pore. In this regard, it has been reported that quinine, quinidine, and chloroquine also block muscle-type nAChRs. Patch-clamp recordings have demonstrated that quinine at micromolar concentrations produces a long-lived open-channel as well as a closed-channel block of muscle nAChRs (Sieb et al., 1996). Similar mechanisms could account for the effects of this drug on α9α10 nAChRs. However, complete analysis at the single-channel level, which so far have been difficult to achieve in α9α10-expressing cells (Plazas et al., 2005), would be necessary to define the underlying mechanism of block.

Quinoline derivatives have a variety of effects on several cochlear hair cell K⁺ currents. Thus, quinine blocks voltage-dependent K⁺ currents in isolated outer hair cells of the guinea pig (Lin et al., 1995). Moreover, it has been proposed that both quinine and quinidine block the small-conductance Ca²⁺-activated K⁺ channels of guinea pig outer hair cells (Yamamoto et al., 1997). Current data support the notion that the inhibitory nature of the cholinergic olivocochlear synapse in both outer and inner hair cells is caused by the activation of a small-conductance Ca²⁺-activated K⁺ current after Ca²⁺ influx through the α9α10-containing nicotinic receptors (Fuchs, 1996; Elgoyhen et al., 2001). The present results show that chloroquine blocks both native and recombinant α9α10-containing nAChRs, thus indicating that quinoline derivatives are direct blockers of the nAChR rather than of the small-conductance Ca²⁺-activated K⁺ channels, as suggested by Yamamoto et al. (1997). Although nonselective for α9α10 nAChRs, the IC₅₀ values obtained for the quinoline derivatives at 10 μM ACh are similar to those obtained for atropine, nicotine, d-tubocurarine, and bicusculine (Elgoyhen et al., 2001). Thus, quinoline derivatives can be used as tools to further define the pharmacological profile of these receptors.

**Clinical Implications.** Quinoline derivatives cause a substantial but usually reversible sensorineural hearing loss at middle to high frequencies accompanied by tinnitus and vertigo (Jung et al., 1993). The mechanism of ototoxicity may involve different levels of the auditory system (Eggermont and Kenmochi, 1998; Jarboe and Hallworth, 1999). However, there is considerable evidence showing that the auditory periphery is the primary location underlying the hearing loss induced by quinine (Puel et al., 1990; Lin et al., 1998). Both a direct action of quinine on the spiral ganglion neurons and/or their synaptic terminals to the inner hair cells and on the outer hair cell electromotility have been proposed as underlying mechanisms for the ototoxicity (McFadden and Pasanen, 1994; Berninger et al., 1998; Zheng et al., 2001). Outer hair cell activity is under the direct control of the efferent cholinergic olivocochlear fibers through the release of ACh and the subsequent activation of α9α10-containing nAChRs (Guinan, 1996; Elgoyhen et al., 2001). The results we present in this work further indicate that these nAChRs are also the target of the quinoline derivatives, and therefore that this effect could eventually contribute to the ototoxicity produced by these compounds. In this regard, it has been shown that chloroquine increases the sensitivity to noise-induced trauma (Barrenas and Holgers, 2000). Because the efferent system protects the inner ear against noise-induced trauma (Patuzzi and Thompson, 1991), block of the system in the presence of chloroquine could account for the above-described increased damage.

Humans receiving treatment with quinoline derivatives can attain plasmatic drug levels within the range in which they block the α9α10 nAChR. Thus, in patients with cardiac arrhythmias and malaria, the therapeutic plasma concentra-

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**Fig. 8.** Effect of chloroquine on ACh-evoked currents in rat cochlear inner hair cells. A, representative traces to ACh either alone or in the presence of chloroquine. B, inhibition curves performed by the coapplication of 60 μM ACh and increasing concentrations of chloroquine. Cells were incubated with each concentration of the antagonist for 2 min before the addition of ACh. Peak current values plotted are expressed as the percentage of the peak control current evoked by ACh. The mean and S.E.M. of five experiments are shown.
tions of quinine and quinidine fall within the micromolar range (Kessler et al., 1974; Franke et al., 1983). Quinine is also used as a flavoring agent in tonic water and some liqueurs (Worner et al., 1989). In the United States and Germany, federal regulations limit the amount of quinine in carbonated beverages to 83 and 85 mg/L, respectively. However, the daily consumption of 105 mg of quinine in tonic water for 2 weeks leads to a serum level of approximately 0.7 μM (Zajtchuk et al., 1984). It is clear that the quinoline derivatives must be used with caution when the safety margin of the auditory system is already compromised.

References


