Inhibition of Volume-Regulated and Calcium-Activated Chloride Channels by the Antimalarial Mefloquine

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ABSTRACT
We have used the whole-cell patch-clamp technique to study the effect of mefloquine (Lariam), a commonly used antimalarial drug, on the volume-regulated anion channel (VRAC) in cultured bovine pulmonary artery endothelial cells. We also examined its effects on other Cl \(^{-}\) channels, i.e., the Ca\(^{2+}\)-activated Cl \(^{-}\) channel and the cystic fibrosis transmembrane conductance regulator, to assess the specificity of this compound for VRAC. At pH 7.4 mefloquine induced a fast and reversible block of the volume-sensitive chloride current (I_{Cl,swell}) with an IC\(_{50}\) value of 1.19 ± 0.07 μM. The blocking efficiency increased with increasing extracellular pH (IC\(_{50}\) value for pH 8.8 was 0.15 ± 0.01 μM), indicating that this effect is mediated by the uncharged form of mefloquine. Ca\(^{2+}\)-activated Cl \(^{-}\) currents, I_{Cl, Ca}, activated by loading T84 cells via the patch pipette with 1 μM free Ca\(^{2+}\) also were inhibited by mefloquine (IC\(_{50}\) value 3.01 ± 0.17 μM at pH 7.4). The cystic fibrosis transmembrane conductance regulator channel, transiently transfected in cultured bovine pulmonary artery endothelial cells, was not affected by 10 μM of the drug. This study describes for the first time effects of mefloquine on anion channels. Our data reveal a potent block of VRAC and Ca\(^{2+}\)-activated Cl \(^{-}\) channel at therapeutic concentrations. These results may contribute to a better understanding of the actions and side effects of this widely used antimalarial drug.

Malaria remains a devastating human infection worldwide, with 300 to 500 million clinical cases and nearly 3 million deaths each year. Much of this mortality is caused by infection with Plasmodium falciparum. Quinoline-containing antimalarial drugs, such as chloroquine (CQ), quinine, and mefloquine, are mainstays of chemotherapy against malaria. The molecular basis of the action of these drugs is not completely understood, but they are thought to interfere with hemoglobin digestion in the blood stages of the malaria parasite’s life cycle. As the malaria parasites become increasingly resistant to quinoline antimalarials, there is an urgent need to understand the molecular mechanisms for drug action and resistance so that novel antimalarial drugs can be designed (Foley and Tilley, 1998).

After infection of human erythrocytes by the malaria parasite, P. falciparum, there is a dramatic increase in the permeability of the red cell membrane to a wide range of low-molecular-weight organic and inorganic solutes, such as monovalent anions and cations, amino acids, monosaccharides, and other polyols, and pyrimidine and purine nucleosides (Kanaani and Ginsburg, 1991; Kirk and Kirk, 1993; Kirk et al., 1994; Kirk and Horner, 1995a,b). Although mainly anion permeable, this pathway also displays a significant cation permeability (Staines and Kirk, 1998; Staines et al., 2000). An apparent function of this transmembrane pathway is to increase the transport capacity for substrates that are used by the intracellular parasite to ensure its growth and maturation and to allow the efflux of toxic compounds. In addition, digestion of hemoglobin by the parasite will produce large quantities of amino acids and increase the amount of osmotically active solute within the infected cell. By mediating the net efflux of amino acids from the red cell, the parasite-induced channel also may regulate the volume of the parasitized red cell. This channel is inhibited by compounds known to block anion channels, such as 5-nitro-2-(3-phenylpropylamino)benzoic acid, furosemide, niflumic acid, and quinine (Kirk and Strange, 1998).

It has been reported previously that the antimalarial quinine potently inhibits volume-regulated anion channels (VRACs) in endothelial cells (Voets et al., 1996b). Mefloquine [Lariam, rac-erythro-α-2-piperidyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol], chemically related to quinine, is a syn-

ABBREVIATIONS: CQ, chloroquine; VRAC, volume-regulated anion channel; MFQ, mefloquine; Ro21, Ro 21-8812/000; Ro14, Ro 14-6858/000; I_{Cl, Ca}, Ca\(^{2+}\)-activated Cl \(^{-}\) current; CACCC, calcium-activated chloride channel; CFT, cystic fibrosis transmembrane conductance regulator; WT, wild type; CPE, cultured bovine pulmonary artery endothelial; GFP, green fluorescent protein; ISO, isotonic solution; HTS, hypotonic solution; IBMX, 3-isobutyl-1-methylxanthine; I_{Cl,swell}, swelling-activated Cl \(^{-}\) current.
thetetic 4-quinoline methanol derivative with two -CF₃ substituents and therefore an interesting candidate for VRAC inhibition (Fig. 1). The drug is widely used in prophylaxis and treatment of CQ-resistant and multidrug-resistant malaria caused by *P. falciparum*. It is a highly effective blood schizontocide, acting on asexual erythrocytic stages of the malarial parasites, but the exact mechanism of action is unknown. The most common side effects associated with mefloquine are neuropsychiatric, gastrointestinal, dermatological, and cardiovascular disorders (Palmer et al., 1993; Tracy and Webster, 1996).

In this study we have tested whether mefloquine (MFQ) and two structural analogs, Ro21-8812/000 [Ro21, 2-pyridyl-2,8-bis(trifluoromethyl)-4-quinoline methanol] and Ro14-6858/000 [Ro14, 2-pyridyl-2,8-bis(trifluoromethyl)-4-quinolylketone] (Fig. 1), modulate VRAC and the corresponding swelling-activated chloride current (I<sub>Cl<sub>swell</sub></sub>). In addition, we also have investigated the effects of CQ (Fig. 1). Many, if not all mammalian cells express VRACs, which are important regulators of various cell functions such as cell volume, pH control, membrane potential, and transport of osmolytes (Nilius et al., 1996, 1997a; Strange et al., 1996; Okada, 1997). It has been demonstrated that these channels are permeable to inorganic anions as well as to various structurally dissimilar organic molecules, such as taurine, myo-inositol, glycine, aspartate, and glutamate (Manolopoulos et al., 1997). These VRACs show functional and pharmacological similarities with the above-mentioned parasite-induced channel and may be an important site of action for MFQ. The rationale was that if MFQ inhibited this channel, in a concentration range that approaches therapeutic concentrations, this knowledge could contribute to a better insight into the actions and side effects of the drug.

In addition, to assess the specificity of the effects on VRAC, we examined whether the Ca<sup>2+</sup>-activated Cl<sup>-</sup> channel (CaCC) and the cystic fibrosis transmembrane conductance regulator (CFTR) also were affected by MFQ. CFTR is a nonrectifying, 8 to 10 pS, protein kinase A-activated chloride channel (Cliff et al., 1992) belonging to the family of ATP-binding cassette proteins (Hyde et al., 1990). CaCC is a strongly outwardly rectifying, 8 pS, calcium-activated chloride channel, described in various excitable and nonexcitable cells, including the human colonic cell line T84 (Valverde et al., 1993).

We show herein that MFQ exerts a potent blocking effect on VRAC at concentrations close to therapeutic concentrations. This suggests that VRAC is a possible site of action for this drug. MFQ also affects CaCC in the same (therapeutic) concentration range, but does not affect CFTR.

**Materials and Methods**

**Vector Construction**

For functional measurements of CFTR, we used the pClNeo/IRES-GFP plasmid (Trouet et al., 1997) for expressing wild-type (WT) CFTR in cultured bovine pulmonary artery endothelial (CPAE) cells. For insertion of WT CFTR the green fluorescent protein (GFP)-vector was cut with EcoRI, dephosphorylated, and thereby unblunt-ended with T4 DNA-polymerase. The WT CFTR cDNA was obtained from a pcDNA/CFTR plasmid through *Sac* I digestion. The fragment obtained was blunt-ended by using T4 DNA-polymerase. Ligation was performed by using standard procedures.

**Cell Culture and Transfection**

**VRAC and CFTR.** Cells from a CPAE cell line (CCL 209; American Type Culture Collection, Manassas, VA) were used. The cells were grown in Dulbecco’s modified Eagle’s medium containing 20% fetal calf serum, 2 mM L-glutamine, 2 μM penicillin, and 2 mg ml<sup>-1</sup> streptomycin. Cultures were maintained at 37°C in a fully humidified atmosphere of 10% CO<sub>2</sub> in air.

Cells were detached by exposure to 0.05% trypsin in a Ca<sup>2+</sup>- and Mg<sup>2+</sup>-free solution, seeded on gelatin-coated coverslips, and kept in culture for 2 to 4 days before use. For electrophysiological experiments, only nonconfluent single endothelial cells were used.

CPAE cells do not express CFTR (Nilius et al., 1997d). Therefore, cells were transiently transfected with WT CFTR in the pClNeo/IRES-GFP vector (Trouet et al., 1997). Briefly, 150,000 cells were incubated with a transfection cocktail containing 3 μg of DNA and 12 μl of polycationic SuperFect Transfection Reagent (Qiagen, Hilden, Germany). Cells were then transferred to gelatin-coated coverslips 24 h after transfection and electrophysiological measurements were done during 2 to 4 days after transfection. Incorporation of WT CFTR in the bicistronic unit allows coupled expression of the channels and GFP. Transfected cells, positive for GFP, could be identified in the patch-clamp setup. GFP was excited at a wavelength between 450 and 490 nm and the emitted light was passed through a 520-nm-long pass filter.

**CaCC.** Cells from a human colon carcinoma (T84) cell line (CCL 248; American Type Culture Collection) were used. The cells were
grown in Dulbecco's modified Eagle's medium/Ham F12 medium containing 5% fetal calf serum, 2 mM L-glutamine, 2 U ml⁻¹ penicillin, and 2 mg ml⁻¹ streptomycin. Cultures were maintained at 37°C in a fully humidified atmosphere of 5% CO₂ in air.

Cells were detached by exposure to 0.05% trypsin in a Ca²⁺- and Mg²⁺-free solution, reseeded on gelatin-coated coverslips, and kept in culture for 2 to 4 days before use. For electrophysiological experiments, only nonconfluent single endothelial cells were used.

**Solutions and Drugs**

The standard extracellular solution was a modified Krebs' solution containing 150 mM NaCl, 6 mM KCl, 1 mM MgCl₂, 1.5 mM CaCl₂, 10 mM glucose, and 10 mM HEPES, titrated with NaOH to pH 7.4. The osmolarity, as measured with a vapor pressure osmometer (Wescor 5500; Schlag, Gladbach, Germany), was 320 ± 5 mOsm.

VRAC. At the beginning of the patch-clamp recording, the Krebs' solution was replaced by an isotonic-Cs⁺ solution (ISO, 320 ± 5 mOsm) containing 150 mM NaCl, 6 mM CsCl, 1.5 mM CaCl₂, 1 mM MgCl₂, 90 mM D-mannitol, 10 mM glucose, and 10 mM HEPES, adjusted to pH 7.4 with NaOH. Volume-sensitive Cl⁻ currents were activated by replacing the cells to a 25% hypotonic extracellular solution (HTS, 240 ± 5 mOsm), containing 105 mM NaCl, 6 mM CsCl, 1.5 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, 10 mM HEPES (HTS₃), or 10 mM Tris(hydroxymethyl)-aminomethane (HTS₄) solution, adjusted to pH 7.4 (HTS₃) or pH 6 (HTS₄) with NaOH or titrated to pH 8.8 (HTS₄) with HCl.

The standard pipette solution contained 40 mM CsCl, 100 mM Cs⁺-aspartate, 1 mM MgCl₂, 1.93 mM CaCl₂, 5 mM EGTA, 4 mM disodium ATP, and 10 mM HEPES, adjusted to pH 7.2 with CsOH (290 ± 5 mOsm). The presence of Cs⁺ instead of K⁺ in the extra- and intracellular solutions blocked the inwardly rectifying K⁺ currents, which are present in CPAE cells (Voets et al., 1996a). To suppress the Cs⁺-activated Cl⁻ current, the free Ca²⁺ concentration in the pipette solution was buffered at 100 nM, which is below the threshold for activation of this current (Nilius et al., 1997b), but which is sufficient for full activation of I_{Cl,w} during cell swelling in CPAE cells (Szücs et al., 1996). The calculated chloride equilibrium potential, E_{Cl,sw}, is -23 mV.

CaCC. Krebs' solution was replaced by a slightly hypertonic Krebs-Cs⁺ solution (345 ± 5 mOsm) containing 150 mM NaCl, 6 mM CsCl, 1 mM MgCl₂, 1.5 mM CaCl₂, 10 mM glucose, 25 mM D-mannitol, and 10 mM HEPES, titrated with NaOH to pH 7.4. The slightly increased osmolality prevented coactivation of VRAC. I_{Cl,sw}, was activated by loading CPAE cells via the patch pipette with 1000 nM free Ca²⁺ as described previously (Nilius et al., 1997b). The standard pipette solution contained 40 mM CaCl₂, 100 mM cesium-aspartate, 1 mM MgCl₂, 4.53 mM CaCl₂, 5 mM EGTA, 4 mM disodium ATP, and 10 mM HEPES, adjusted to pH 7.2 with Ca(OH)₂ (290 ± 5 mOsm). The presence of Cs⁺ instead of K⁺ in the extra- and intracellular solutions blocked the inwardly rectifying K⁺ currents, which are present in CPAE cells (Voets et al., 1996a). To suppress the Cs⁺-activated Cl⁻ current, the free Ca²⁺ concentration in the pipette solution was buffered at 100 nM, which is below the threshold for activation of this current (Nilius et al., 1997b), but which is sufficient for full activation of I_{Cl,w} during cell swelling in CPAE cells (Szücs et al., 1996). The calculated chloride equilibrium potential, E_{Cl,sw}, is -23 mV.

**Current Measurements and Data Analysis**

Whole-cell membrane currents were measured in ruptured patches. All experiments were performed at room temperature (20–23°C). Currents were monitored with an EPC-7 patch-clamp amplifier (List Electronic, Darmstadt, Germany) and sampled at 2-ms intervals (1024 points/record, filtered at 200 Hz), unless otherwise mentioned. Patch electrodes had a resistance between 3 and 5 MΩ. An Ag-AgCl wire was used as reference electrode.

**VRAC and CaCC.** In most experiments we applied a "ramp" protocol, which consisted of a step to -80 mV for 0.4 s, followed by a step to -150 mV for 0.1 s and a 1.3-s linear voltage ramp to +100 mV. This voltage protocol was repeated every 15 s from a holding potential of -20 mV. Current-voltage relations were constructed from the ramp current, and time courses were obtained from the current at +100 mV and -150 mV. In some experiments we used a "step" protocol consisting of 1-s voltage steps, applied every 2 s from a holding potential of -20 mV (VRAC) or -50 mV (CaCC) to test potentials from -100 to +100 mV with increments of 20 mV. Currents were sampled at 1-ms intervals.

**CFTR.** In most experiments, a "ramp" protocol was used, consisting of 400-ms linear voltage ramps from -100 mV to +100 mV, repeated every 10 s from a holding potential of -20 mV. Currents were sampled at 0.5-ms intervals and filtered at 5 kHz. The time course was obtained from the current at +100 and -100 mV. In some experiments we used a "step" protocol consisting of 1-s voltage steps, applied successively from a holding potential of -20 mV. Data were analyzed in Winascd (by G. Droogmans) and in Origin (MicroCal Software, Inc., Northampton, MA). Pooled data are given as the mean ± S.E.

**Results**

**Mefloquine Inhibits VRAC and CaCC, but Not CFTR.**

Ca²⁺-activated Cl⁻ currents, I_{Cl,sw}, were activated by loading T84 cells via the patch pipette with 1 μM free Ca²⁺ as described previously (Nilius et al., 1997c). Figure 2, A and B, shows current traces in control conditions and in the presence of 5 μM MfQ, measured during the voltage step protocol, performed at pH 7.4. The corresponding current-voltage relations are shown in Fig. 2C.

CFTR currents were activated by a cocktail containing 100 μM IBMX and 1 μM forskolin dissolved in the Krebs-Cs⁺ solution, as described in detail elsewhere (Cuppens et al., 1998). Figure 2, D and E, shows CFTR current traces in control conditions and in the presence of 10 μM MfQ. The corresponding current-voltage relations (Fig. 2F) show that 10 μM MfQ does not significantly affect the CFTR currents. The results of six cells are depicted in Table 1. Volume-activated chloride channels and the corresponding current, I_{Cl,sw}, were activated by replacing the ISO by the HTS, as described in detail elsewhere (Nilius et al., 1994). Figure 2, G and H, shows current traces in control conditions and in the presence of 2 μM MfQ, measured during the voltage step protocol, performed at pH 7.4. The corresponding current-voltage relations (Fig. 2I) show that the VRAC currents are approximately equally inhibited at positive and negative potentials.

**Inhibition by MFQ of CaCC.** I_{Cl,sw} differs in many aspects from I_{Cl,sw}. The outward rectification is much more pronounced than that of I_{Cl,sw} and the kinetic behavior of both currents is completely different (Nilius et al., 1997b). Figure 3A shows a typical time course of this current, activated by loading the T84 cell with 1 μM free calcium. The current indicated by "a" is measured just after breaking into
the cell, i.e., before the intracellular Ca$^{2+}$ concentration has risen enough to activate the CaCC, and represents the background current. The inhibitory effect of 5 µM MFQ on the fully activated $I_{Cl,Ca}$ is shown. Figure 3B depicts the corresponding current-voltage relations. The concentration dependence of the MFQ inhibition of CaCC was further investigated. The dose-inhibition curve (Fig. 3C), at +100 mV and derived from responses at five different concentrations, was fitted to the following equation:

\[
\text{% Inhibition} = \frac{100}{1 + (IC_{50}/C)^p}
\]

in which C is the drug concentration, p the Hill coefficient, and IC$_{50}$ the drug concentration needed for half-maximal block. At pH 7.4, the estimated value of IC$_{50}$ value was 3.01 ± 0.17 µM, with a Hill coefficient of 1.0 ± 0.1 (n = 4–8).

We compared the effects of 10 µM MFQ and its structural analogs Ro14 and Ro21. Although 10 µM MFQ almost completely inhibited $I_{Cl,Ca}$, 10 µM Ro21 only caused small current inhibition, and 10 µM Ro14 did not significantly affect the current (Table 1).

**Inhibition by MFQ of VRAC.** At pH 7.4, MFQ exists primarily with one single positive charge, due to the piperidine ring, with a pK$_a$ value of 9. To investigate whether the inhibitory effect of MFQ on $I_{Cl,swell}$ is mediated by the posi-

**Table 1** Effects of antimalarials on three different Cl$^-$ currents

<table>
<thead>
<tr>
<th>Current</th>
<th>Treatment</th>
<th>% Inhibition</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>VRAC MFQ</td>
<td>10 µM</td>
<td>93.32 ± 2.58$^a$</td>
<td>9</td>
</tr>
<tr>
<td>Ro14</td>
<td>1.72 ± 1.05$^b$</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Ro21</td>
<td>29.01 ± 5.38$^a$</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>CQ</td>
<td>1.21 ± 1.21$^b$</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>CaCC MFQ</td>
<td>75.82 ± 2.41$^a$</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Ro14</td>
<td>3.49 ± 3.76$^a$</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Ro21</td>
<td>7.18 ± 2.61$^b$</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>CFTR MFQ</td>
<td>3.53 ± 7.02$^b$</td>
<td>6</td>
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$^a$ Calculated from current measured at +100 mV when complete inhibition was reached.

$^b$ Calculated from current measured at +100 mV after at least 2 min in the presence of the drug.
tively charged or by the neutral form, we performed experiments in which the pH of the HTS solution was increased to 8.8 (HTS8.8) or decreased to 6.0 (HTS6) during drug application. Short (1–2 min) changes in extracellular pH only do not have substantial effects on the instantaneous current amplitude. Nilius et al. (1998) showed that a decrease of extracellular pH from 7.4 to 6.0 slightly reduced the size of the current by 15\% whereas a change from pH 7.4 to 9.0 only induced a minimal current increase of 9\%. In our experiments, the increase in the current at basic pH would even be less because we changed the pH value only to 8.8 instead of 9. Taking these findings into consideration, we may slightly overestimate the inhibition at pH 6 and even underestimate it at pH 8.8. Figure 4A shows a typical time course experiment in which the pH of the external solution was changed during application of 2 μM MFQ. The current decrease due to pH 6 is around 15\%. The current trace at time a represents the “background” current, before the activation of I_{Cl,swell}. Current-voltage relationships (Fig. 4B) are obtained from voltage ramps in control conditions and in the presence of 2 μM MFQ at different pH values. Figure 4 shows that the inhibitory effect of 2 μM MFQ on I_{Cl,swell} is increased at higher pH and significantly reduced at lower pH values. The inhibition by 2 μM MFQ at different pH values measured in different cells was 14.55 ± 2.57% for pH 6 (n = 6), 65.49 ± 2.72% for pH 7.4 (n = 9), and 95.65 ± 1.84% for pH 8.8 (n = 9). The inhibition observed at pH 6 is, at least partly, due to the change of the pH. We have disregarded the minimal underestimation of the inhibitory effect at pH 8.8.

We have evaluated the concentration dependence of the inhibition of I_{Cl,swell} for MFQ at two pH values (pH 7.4 and 8.8) and at +100 mV. The inhibition is expressed as the percentage reduction of the background corrected I_{Cl,swell} at +100 mV. The background current (current under isotonic conditions) was not affected by MFQ. The dose-inhibition curve, depicted in Fig. 5, was fitted to eq. 1. The IC_{50} value and Hill coefficient were 1.19 ± 0.07 μM and 1.2 ± 0.1,
respectively, at pH 7.4, and 0.15 ± 0.01 μM and 1.4 ± 0.1 at pH 8.8. This more potent block at higher pH could be explained by assuming that mainly the uncharged form of MFQ exerts a blocking effect. The uncharged concentrations can be calculated by the following equation:

\[
\frac{[\text{Base}]}{[\text{MFQ}]} = \frac{10^{0.1\cdot pK_a}}{1 + 10^{0.1\cdot pK_a}}
\]

(2)

In the case of MFQ, [Base] and [MFQ] correspond to the concentration of the uncharged drug and total drug concentration, i.e., the sum of the charged and uncharged forms of MFQ. It can be calculated that at pH 6 only 0.10% of MFQ is in its uncharged form, whereas at pH 7.4 and 8.8 the uncharged form amounts to 2.45 and 38.68%, respectively. Hence, a pH shift from pH 7.4 to 8.8 increases the concentration of the uncharged form approximately 15-fold. The ratio of the IC_{50} values at pH 7.4 and 8.8 amounts to 7.9 and 38.68%, respectively. Hence, a pH shift from pH 7.4 to 8.8 increases the concentration of the uncharged form approximately 15-fold. The ratio of the IC_{50} values at pH 7.4 and 8.8 amounts to 7.9 and 38.68%, respectively.

The inhibitory effect of MFQ on VRAC is markedly potentiated at higher pH values. The pronounced pH effect is not due to an effect on the channel itself. First, short changes in extracellular pH influence the kinetic behavior of I_{Cl,swell}, but do not substantially affect the instantaneous current amplitude. Second, it has been shown that increasing the extracellular pH from 7.4 to 8.8 slightly increases the current (Nilius et al., 1998). At the composite current, this would counteract the observed inhibition. Our results therefore strongly suggest that the uncharged form of MFQ mainly mediates inhibition of VRAC. A possible explanation could be that the block is due to an intracellular action after permeation of the uncharged form through the cell membrane, as has been proposed for chromones (Heinke et al., 1995). However, if uncharged MFQ enters the cell, it would be ionized immediately (the internal solution has a pH of 7.2) and subsequent washout would be rather slow. Because this mechanism is not consistent with our results, it is more likely that the high-affinity block by the neutral MFQ is due to hydrophobic interactions with the channel protein(s) within the membrane bilayer. The voltage-independent nature of the block further supports this hypothesis. If we assume that only the nonionized form of MFQ is responsible for block of VRAC, the estimated IC_{50} value is 47.6 ± 1.4 nM as derived from the measurements at pH 7.4 and 8.8. A similar mechanism has been described for the inhibitory action of quinine on I_{Cl,swell} with a predicted IC_{50} value of 67 ± 13 nM for the uncharged form (Voets et al., 1996b).

Several studies showed that infections with the malaria parasite *P. falciparum* induce a permeation pathway in human erythrocytes that seems to be important for the survival of the parasite (Kirk and Kirk, 1993; Kirk et al., 1994; Kirk and Horner, 1995a,b). In addition to playing a likely role in nutrient uptake and waste excretion, the parasite-induced channel also may regulate the volume of the parasitized red cell (Kirk and Strange, 1998). It has been shown that a variety of anion channel blockers, including 5-nitro-2-(3-phenylpropylamino)benzoic acid and analogs, inhibit this channel and a number of these have been tested for their effect on the growth of the malaria parasite in vitro. In all cases, compounds that inhibit the channel inhibit the growth of the parasite (Kirk and Horner, 1995a). The pharmacological and selectivity characteristics of this channel are very similar to VRACs described in many cell types (Strange et al., 1996; Nilius et al., 1997a; Okada, 1997). Patch-clamp experiments revealed novel channel activity in the parasitized erythrocyte (Desai et al., 1996), but the detailed electrophysiological characteristics of the parasite-induced anion channel remain to be established (Kirk and Strange, 1998). Therefore, although not yet unambiguously established, VRAC seems to be a promising candidate for this channel. Therefore, the herein-described block of VRAC by MFQ could at least partly explain the antimalarial action of this drug. An interesting observation is that the IC_{50} for quinine-induced inhibition of VRAC of 20 μM (Voets et al. 1996b) matches well the therapeutically relevant concentrations, which range from 25 to 46 μM (Webster, 1990). This also could provide a possible working

**Discussion**

Our results demonstrate a fast and concentration-dependent block of VRACs by MFQ. This effect was not specific for VRAC because CaCC also was inhibited in a similar concentration range. CFTR was not affected. These results may contribute to a better insight into the actions and side effects of the antimalarial drug MFQ because the effects occur at therapeutically relevant concentrations. Therapeutic plasma levels range from 1.5 to 3.3 μM (Hellgren et al., 1990; Goldsmith, 1995), with apparently higher brain concentrations (Pham et al., 1999).
The inhibition of IC\(_{\text{Cl,swell}}\) we also observed an inhibition of IC\(_{\text{Cl,Ca}}\) at 10-fold higher than the therapeutic plasma concentrations (i.e., 0.4–0.8 \(\mu\)M; Webster, 1990) does not inhibit VRAC is not necessarily in contradiction with this proposed working mechanism because both mefloquine and quinine are effective in (and reserved for) treatment of infection with CQ-resistant strains of \(P.\) \textit{falciparum} (Goldsmith, 1995). Considering that the mechanism of quinoline-containing antimalarials has not yet been elucidated and that there seems to exist substantial differences between the quinoline-containing antimalarials, it is not unlikely that they have different and perhaps several mechanisms of action (Tracy and Webster, 1996). Furthermore, it has been suggested that CQ-resistant parasites appear to expel CQ via a membrane P-glycoprotein pump similar to that described for multidrug-resistant cancer cells (Goldsmith, 1995). Because this is not the case for the two other quinoline-containing antimalarials mefloquine and quinine, there must be substantial structural differences between on the one hand CQ, and on the other hand mefloquine and quinine.

The use of MFQ as a prophylactic or curative antimalarial drug has been associated with relatively frequent reports of mild central nervous system toxicity. Administration of MFQ can result in symptoms of confusion, dizziness, and dysphoria, and occasionally in severe neuropsychiatric effects (Bem et al., 1992; Palmer et al., 1993; Hennequin et al., 1994). Although these occasional side effects are considered acceptable during the treatment of life-threatening severe malaria, they have compromised the usefulness of MFQ as a prophylactic drug. In addition, other more common side effects also have been reported (Palmer et al., 1993). It has been shown that VRAC is a ubiquitously expressed channel with very similar properties in many cell types. The functional importance of this channel has been discussed in detail and comprises among others, effects on volume regulation, osmolyte transport, and electrogenesis (Strange et al., 1996; Nilius et al., 1997a; Okada, 1997). Because of these multiple effects, it is not unlikely that modulation of VRAC, at least in part, can result in symptoms of confusion, dizziness, and dysphoria. Therefore, it was interesting to study the effects of MFQ not only on VRAC but also on CaCC and CFTR. We demonstrated that both VRAC and CaCC are inhibited by MFQ, with IC\(_{50}\) values in the same order of magnitude, i.e., 1.2 and 3.0 \(\mu\)M, respectively. CFTR was not affected by 10 \(\mu\)M MFQ.

From a structural point of view, it is interesting to compare MFQ with quinine and with its structural analogs Ro14 and Ro21 (Fig. 1). In comparison with quinine, MFQ lacks the ethylene bridge on the piperedine and an acetyl group on the quinoline. The latter was substituted with two -CF\(_3\) groups in positions 2 and 8. If we assume that principally the uncharged form is responsible for VRAC inhibition, we need to compare the estimated IC\(_{50}\) values for this form, i.e., 67 nM for quinine and 48 nM for MFQ, indicating that the above-mentioned modifications enhance the inhibition by approximately 30%. Ro14 and 21 differ from MFQ only in the piperedine, which is replaced by a pyridine and therefore reduces the basicity, with Ro21 being more basic than Ro14. Taking this into account, the amount of the uncharged form of these compounds at the same total concentration will increase in the order MFQ < Ro21 < Ro14. This order is opposite to the observed blocking potency of these compounds, suggesting that the piperedine determines the blocking potency. This is confirmed by the fact that CQ contains only the quinoline, but no piperedine. To find more potent blockers, further modifying the MFQ molecule, especially the position and number of -CF\(_3\) groups on the quinoline, would be interesting. In addition to this, it would be useful to investigate the possible enantioselectivity of the interactions, because Lariam and thus MFQ, is used as a racemic mixture.

In conclusion, VRACs and CaCCs are efficiently blocked by MFQ at therapeutically relevant concentrations. This information may contribute to a better insight into the actions and side effects of this widely used antimalarial drug. In addition, the knowledge of this possible mechanism and the pharmacological profile of VRACs could offer completely new strategies to inhibit the growth of the malaria parasites.

Acknowledgments

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References


Hellgren U, Angel VH, Bergqvist Y, Arvidsson A, Forero Gomez JS and Rembo L (1990) Plasma concentrations of sulfadoxine-pyrimethamine and of mefloquine...


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