Blockade of Currents by the Antimalarial Drug Chloroquine in Feline Ventricular Myocytes

JOSÉ A. SÁNCHEZ-CHAPULA, EDUARDO SALINAS-STEFANON, JULIAN TORRES-JÁCOME, DORA E. BENAVIDES-HARO, and RICARDO A. NAVARRO-POLANCO

Unidad "Carlos Méndez" del Centro Universitario de Investigaciones Biomédicas de la Universidad de Colima, Colima, México (J.A.S.-C., D.E.B.-H., R.N.-P.); and Instituto de Fisiología de la Benemérita Universidad Autónoma de Puebla, Puebla, México (E.S.-S., J.T.-J.)

Received September 15, 2000; accepted December 13, 2000

This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

The effects of the antimalarial drug chloroquine on cardiac action potential and membrane currents were studied at clinically relevant concentrations. In cat Purkinje fibers, chloroquine at concentrations of 0.3 to 10 μM increased action potential duration, and reduced maximum upstroke velocity. At concentrations of 3 and 10 μM, chloroquine increased automaticity and reduced maximum diastolic potential, and after 60 min of perfusion with a concentration 10 μM, spontaneous activity was abolished. In isolated cat ventricular myocytes, chloroquine also increased action potential duration in a concentration-dependent manner, and reduced resting membrane potential at 3 and 10 μM. In voltage-clamped cat ventricular myocytes, chloroquine blocked several inward and outward membrane currents. The order of potency was inward rectifying potassium current (I_K1) > rapid delayed rectifying potassium current (I_Kr) > sodium current (I_Na) > L-type calcium current (I_Ca-L). Only tonic block of I_Na and I_Ca-L was observed at a stimulation frequency of 0.1 Hz and no additional blockade was observed during stimulation trains applied at 1 Hz. The effect of chloroquine on I_K1 was voltage-dependent, with less pronounced blockade at negative test potentials. In addition, unblock was achieved by hyperpolarizing pulses to potentials negative to the current reversal potential. Chloroquine blocked the rapid component of the delayed rectifying outward current, I_Kr, but not the slow component, I_KS. These findings provide the cellular mechanisms for the prolonged QT interval, impaired ventricular conduction, and increased automaticity induced by chloroquine, which have been suggested as responsible for the proarrrhythmic effects of the drug.

Malaria remains one of the most important and widespread diseases in the world. Chloroquine is one of the drugs of first choice for treatment of malaria. Chloroquine is also used as an anti-inflammatory agent in rheumatoid arthritis and in lupus erythematosus (Webster, 1992). However, the use of chloroquine has been associated with toxic cardiovascular effects, including a fall in blood pressure (Olatunde, 1970) and rhythm abnormalities (Williams, 1966; Guedira et al., 1998). Prolonged therapy can lead to cardiac failure (Hughes et al., 1971) and electrocardiographic changes, including T-wave depression or inversion, and prolonged QRS and QTc intervals (Sanghvi and Mathur, 1965; Bustos et al., 1994). Chloroquine has also been reported to induce torsade de pointes (Harris et al., 1988; Fauchier et al., 1993), a tachyarrhythmia associated with medications that block repolarizing cardiac K⁺ currents. Acute poisoning by chloroquine can cause death by failure of myocardial contraction and cardiac arrest (Don-Michael and Aiwazzadeh, 1970).

The proarrrhythmic effects of chloroquine are well documented; however, there are only two published studies describing the cellular electrophysiological effects of this drug. Harris et al. (1988) used microelectrode techniques and multicellular preparations (sheep ventricular muscle and Purkinje fibers) to demonstrate that 5 to 50 μM chloroquine produced significant reduction in the maximum upstroke velocity (V_max) of the action potential, an indirect measure of peak sodium channel conductance. Harris et al. (1988) also demonstrated that chloroquine prolonged action potential duration and refractory period, effects usually attributed to block of K⁺ currents. Recently, Benavides-Haro and Sanchez-Chapula (2000) demonstrated that chloroquine blocked inward rectifier current (I_K1) and the acetylcholine K⁺ current, I_K(Ach), in guinea pig atrial and ventricular myocytes.

A slowing in ventricular conduction and an excessive lengthening in the QT interval have been proposed as the

ABBREVIATIONS: V_max, maximum upstroke velocity; I_K1, inward rectifying potassium current; I_Na, sodium current; I_Ca-L, type L calcium current; I_TO, transient outward potassium current; I_KD, rapid delayed rectifying potassium current; I_KS, slow delayed rectifying potassium current; APD, action potential duration; I_p, pacemaker current.
mechanisms of the proarhythmic effects of chloroquine (Harris et al., 1988; Bustos et al., 1994; Guedira et al., 1998). Blockade of inward sodium current (\(I_{\text{Na}}\)) has been suggested as the principal cause of impaired ventricular conduction, and acquired long QT syndrome is usually caused by blockade of one or more potassium currents (Nattel, 1998). In the present study, we investigated the effects of chloroquine on action potentials and the major ionic currents contributing to the shape of the action potential in isolated feline ventricular myocytes. Chloroquine lengthened action potentials of cat Purkinje fibers, and increased automaticity. Standard voltage-clamp techniques were used to record \(I_{\text{Na}}\) and L-type calcium current (\(I_{\text{Ca,L}}\)), and four potassium currents, including the \(I_{\text{K1}}\), the transient outward current (\(I_{\text{to}}\)), and the rapid and slow delayed rectifier outward currents (\(I_{\text{Kr}}\) and \(I_{\text{Ks}}\)). Chloroquine blocked four of these currents: \(I_{\text{K1}}, I_{\text{Kr}}, I_{\text{Na}},\) and \(I_{\text{Ca,L}}\). These findings provide the cellular mechanism for the prolonged action potentials and reduction in \(V_{\text{max}}\) of cardiac action potentials previously reported by Harris et al. (1988), and insights into the mechanism of induction of arrhythmias.

**Materials and Methods**

**Standard Microelectrode Technique.** Adult cats (2–4 kg) were anesthetized with sodium pentobarbital (35 mg/kg) and heparinized (1000 U/kg). Free-running Purkinje strands were obtained from the left ventricle of the cat hearts. The Purkinje strands were fixed to the Sylgard (Dow Corning Co., Midland, MI)-coated bottom of a Plexiglas chamber (2-ml volume) with micropins. The preparations were superfused with a solution containing 125 mM NaCl, 24 mM NaHCO\(_3\), 0.43 mM NaH\(_2\)PO\(_4\), 4 mM KCl, 1.8 mM CaCl\(_2\), 1.05 mM MgCl\(_2\), and 11 mM glucose. The solution was equilibrated with 95% O\(_2\), 5% CO\(_2\) (pH 7.4). Temperature was kept constant at 35°C. The preparations were allowed to equilibrate for 60 min before experimental protocols were performed. During this time the preparations were stimulated at a frequency of 1 Hz with rectangular stimuli (3-ms duration, 1.5 times diastolic threshold intensity) delivered by insulated (except at the tips) silver bipolar electrodes. Action potentials were recorded by using glass microelectrodes filled with 3 M KCl (resistance 10–15 MΩ) coupled to the input of a high-impedance preamplifier (World Precision Instruments, New Haven, CT). Action potential signals were digitized at a sampling rate of 10 kHz by use of an analog-to-digital converter (Digidata 1200 interface; Axon Instruments, Burlingame, CA) and stored on a hard disk, Axotape data-acquisition software (Axon Instruments), and a 486DX2 computer. Data analysis was performed using pClamp software (version 6.0.4; Axon Instruments). We have performed experiments recording action potentials in Purkinje fibers for up to 8 h under control conditions. We have not observed significant changes in action potential parameters or spontaneous firing frequency.

**Cell Preparation.** Single ventricular myocytes were obtained from the right ventricular free wall of adult cats as previously described (Sanchez-Chapula, 1996). The hearts were mounted on a Langendorff apparatus and perfused for 5 min with normal Tyrode's solution, and then switched to a nominally calcium-free solution for an additional 5 min. Afterward, the hearts were perfused for 30 min with a zero-calcium solution containing 1 mg/ml type I collagenase (Sigma Chemical Co., St. Louis, MO) and 0.05 mg/ml protease XIV (Sigma Chemical Co.). The enzymes were washed out by perfusion with a high-potassium, low-chloride saline (KB medium; Isenberg and Klockner, 1982) for 5 min. The free wall of the right ventricle was dissected away from the rest of the heart and cut into small pieces. Single cells were maintained in a high-potassium, low-chloride solution at 4°C for up to 10 h before use in electrophysiological experiments.

**Electrical Recordings.** A few drops of the cell suspension were placed in a chamber (0.5-ml volume) mounted on a modified stage of an inverted microscope (Nikon Diaphot, Tokyo, Japan). The chamber was superfused at a rate of 0.5 ml/min with normal external solution. Action potential experiments in isolated ventricular myocytes were performed at 35°C, using the whole-cell “perforated patch” current clamp technique. Sodium, calcium, and potassium currents were recorded using the whole-cell standard patch-clamp method (Hamill et al., 1981) and an Axopatch 1C patch-clamp amplifier (Axon Instruments). A Labmaster-TL/1 interface (Axon Instruments) controlled by pClamp 6.0.3 software (Axon Instruments) was used to generate voltage-clamp command protocols and acquire data. Currents were filtered at 2 kHz with a four-pole Bessel filter, digitally sampled at 4 kHz and stored on the hard disk of an Epson 486Dx/33 computer. \(I_{\text{K}}\) currents were recorded at a sampling frequency of 2 kHz and filtered at 1 kHz. Micropipettes were pulled from borosilicate glass capillary tubes (TW 150-6; World Precision Instruments, Inc., Sarasota, FL) on a programmable horizontal puller (Sutter Instruments, Novato, CA). When filled with the intracellular solution, the pipette tip resistance was 1 to 2 MΩ. Series resistance compensation was set to 80%, and whole-cell capacitance compensation was optimized to minimize capacitative currents and reduce voltage errors. We only analyzed experiments in which access resistance was ≤1.2 MΩ after compensation. The experiments to determine the effect of chloroquine on \(I_{\text{Na}}\) were performed at a temperature of 15°C.

**Fig. 1.** Effect of chloroquine on action potential. A, action potentials recorded from a Purkinje fiber during stimulation at 1 Hz, using standard microelectrode techniques, under control conditions and in the presence of chloroquine 1 and 10 \(\mu\)M. B, effect of chloroquine 1 and 10 \(\mu\)M on a ventricular myocyte action potential recorded during stimulation at 1 Hz, using the perforated patch technique.
Recordings of calcium and potassium currents were performed at 35°C.

**Solutions.** Tyrode’s solution had the following composition: 125 mM NaCl, 24 mM NaHCO₃, 0.42 mM NaH₂PO₄, 5.4 mM KCl, 1.8 mM CaCl₂, 1.05 mM MgCl₂, 11 mM glucose, and 10 mM taurine. The solution was equilibrated with 95% O₂, 5% CO₂, pH 7.4. Nominally, calcium-free solution was prepared by omitting CaCl₂ from the Tyrode’s solution. The high-potassium, low-chloride solution (KB medium) had the following composition: 80 mM potassium glutamate, 50 mM KCl, 20 mM taurine, 3 mM KH₂PO₄, 10 mM glucose, 10 mM HEPES, and 0.2 mM EGTA. The pH was adjusted to 7.4 with KOH.

The normal external solution used to record action potentials in isolated ventricular myocytes had the following composition: 140 mM NaCl, 4 mM KCl, 1.8 mM CaCl₂, 1.0 mM MgCl₂, 10 mM HEPES, and 11 mM glucose, pH adjusted to 7.4 with NaOH. The pipette solution had the following composition: 90 mM potassium aspartate, 45 mM KCl, 10 mM KH₂PO₄, 5 mM HEPES, and 200 μg/ml nystatin, pH 7.3 with KOH.

For measuring INa, the external solution contained 10 mM NaCl, 120 mM CsCl, 0.5 mM CaCl₂, 2 mM MgCl₂, 10 mM HEPES, and 11 mM glucose; pH was adjusted to 7.4 with CsOH. The pipette solution was composed of 132 mM CsCl, 8 mM NaCl, 5 mM MgATP, 5 mM HEPES, and 5 mM EGTA; pH was adjusted to 7.3 with CsOH.

For measuring ICa-L, the external solution contained 140 mM tetraethylammonium chloride, 4 mM CsCl, 3.6 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, and 11 mM glucose; pH was adjusted to 7.4.

**Fig. 2.** Effect of chloroquine on automaticity in cat Purkinje fibers. Spontaneous action potential firing under control conditions and in the presence of chloroquine (1–10 μM).

**TABLE 1**
Concentration-dependent effects of chloroquine on action potential parameters of cat Purkinje fibers and isolated cat ventricular cells

<table>
<thead>
<tr>
<th></th>
<th>MDP</th>
<th>AMP</th>
<th>Vmax</th>
<th>APD₅₀</th>
<th>APD₉₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mV</td>
<td>mV</td>
<td>V/s</td>
<td>ms</td>
<td>ms</td>
</tr>
<tr>
<td>Purkinje fibers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-88±1</td>
<td>127±3</td>
<td>465±28</td>
<td>213±11</td>
<td>313±21</td>
</tr>
<tr>
<td>Chloroquine 0.3 μM</td>
<td>-88±1</td>
<td>127±3</td>
<td>457±24</td>
<td>232±10</td>
<td>345±18*</td>
</tr>
<tr>
<td>Chloroquine 1 μM</td>
<td>-87±2</td>
<td>124±3</td>
<td>418±34</td>
<td>245±12*</td>
<td>359±23*</td>
</tr>
<tr>
<td>Chloroquine 3 μM</td>
<td>-85±2</td>
<td>120±3</td>
<td>287±41*</td>
<td>272±16*</td>
<td>409±29*</td>
</tr>
<tr>
<td>Chloroquine 10 μM</td>
<td>-80±3*</td>
<td>111±4*</td>
<td>111±21*</td>
<td>248±21*</td>
<td>488±31*</td>
</tr>
<tr>
<td>Ventricular cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-86±2</td>
<td>137±4</td>
<td>134±5</td>
<td>157±5</td>
<td></td>
</tr>
<tr>
<td>Chloroquine 0.3 μM</td>
<td>-86±2</td>
<td>137±4</td>
<td>140±5</td>
<td>169±5*</td>
<td></td>
</tr>
<tr>
<td>Chloroquine 1 μM</td>
<td>-85±2</td>
<td>135±4</td>
<td>141±5</td>
<td>174±6*</td>
<td></td>
</tr>
<tr>
<td>Chloroquine 3 μM</td>
<td>-84±2</td>
<td>130±5</td>
<td>138±5</td>
<td>179±7*</td>
<td></td>
</tr>
<tr>
<td>Chloroquine 10 μM</td>
<td>-81±2*</td>
<td>122±6*</td>
<td>129±6</td>
<td>189±8*</td>
<td></td>
</tr>
</tbody>
</table>

* Statistically significant (p < 0.05).
with tetraethylammonium hydroxide. The internal solution had the following composition: 140 mM CsCl, 5 mM MgATP, 5 mM HEPES, and 5 mM EGTA; pH was adjusted to 7.3 with KOH.

Chloroquine-HCl (Sigma Chemical Co.) was directly dissolved in the different external solution at the desired concentration. MK-499 (kindly provided by Dr. J. J. Lynch, Jr., Merck & Co., Inc., West Point, PA) was dissolved in dimethyl sulfoxide as a 0.1 M stock solution. Nystatin was dissolved in dimethyl sulfoxide at a concentration of 25 mg/ml.

Statistics. Data are expressed as means ± S.E.M. Statistical significance was evaluated by ANOVA and Dunnett’s t test. Differences were considered significant at P < 0.05.

Results

Effects of Chloroquine on Action Potentials. Chloroquine increased action potential duration and decreased V max in a concentration-dependent (0.3–10 μM) manner in cat Purkinje fibers stimulated at a basic cycle length (BCL) of 1000 ms (Fig. 1A; Table 1). In addition, at concentrations of 3 and 10 μM, chloroquine decreased the maximum diastolic potential. Action potentials in ventricular myocytes were elicited by 5-ms pulses applied at a basic cycle length of 1000 ms, using the whole-cell perforated patch-clamp technique. Chloroquine at concentrations 1 to 10 μM increased action potential duration (APD) in a concentration-dependent manner (Fig. 1B; Table 1). At concentrations 3 and 10 μM, it decreased resting membrane potential, action potential peak, and plateau amplitudes (Fig. 1B; Table 1).

When external stimulation was discontinued under control conditions, three of five cat Purkinje fibers showed a spontaneous firing frequency of 0.28 Hz; the other two preparations became quiescent. Chloroquine increased firing frequency in all five preparations in a concentration-dependent manner (Fig. 2). However, after 60 min of superfusion with chloroquine, spontaneous activity was abolished in four of five preparations at a resting membrane potential of −46 ± 3 mV. Chloroquine did not induce afterdepolarizations at any of the concentrations used (n = 5).

Effects of Chloroquine on I Na. To improve voltage control, the experiments were performed at 15°C using a low-sodium external solution (under Materials and Methods). In patch-clamp experiments, the voltage dependence of I Na activation and inactivation slowly drifts toward more negative potentials during the first 10 min after rupture of the membrane patch (Kimitsuki et al., 1990). In the present study, control INa recordings were initiated at least 15 min after the rupture of the membrane patch. To test the effects of chloroquine (0.3–10 μM) on I Na, 40-ms depolarizing pulses were applied from a holding potential of −120 mV to membrane potentials ranging from −70 to +10 mV, obtained under control conditions. B, current traces obtained in the presence of chloroquine 10 μM. C, I-V relationships for the peak current amplitude recorded under control conditions, and in the presence of chloroquine, mean ± S.E. of n = 5 cells are shown.

Fig. 3. Effect of chloroquine on I Na in cat ventricular myocytes. From a holding potential of −120 mV, test pulses to different membrane potentials were applied at a frequency of 0.1 Hz. A, current traces induced by test pulses to membrane potentials ranging from −70 to +10 mV, obtained under control conditions. B, current traces obtained in the presence of chloroquine 10 μM. C, I-V relationships for the peak current amplitude recorded under control conditions, and in the presence of chloroquine, mean ± S.E. of n = 5 cells are shown.
was studied by applying trains of 20-ms pulses from a holding potential of −120 mV to a test potential of +20 mV. No significant use-dependent effects were observed (data not shown).

**Effects of Chloroquine on I_{Ca-L}**

The experiments on calcium and potassium currents were performed at 35°C. The effect of chloroquine on I_{Ca-L} was studied by applying depolarizing pulses to membrane potentials ranging from −40 to +40 mV from a holding potential of −70 mV. Pulses were applied at a frequency of 0.1 Hz. Chloroquine at 10 μM decreased the peak amplitude of I_{Ca-L} measured at +10 mV by 32 ± 11% (n = 5) (Fig. 4). The drug did not alter the shape of the I_{Ca-L}-V relationship (Fig. 4). The effect of chloroquine on I_{Ca-L} at a test potential of +10 mV was concentration-dependent (Table 2). Each myocyte was treated with a single concentration of chloroquine. The effects of chloroquine at all

---

**TABLE 2**

Concentration-dependent effects of chloroquine on different ionic currents

<table>
<thead>
<tr>
<th>Current</th>
<th>Drug Concentration (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>I_{Na}</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>I_{Ca-L}</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>I_{K1}</td>
<td>*51 ± 10</td>
</tr>
<tr>
<td>I_{to}</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>I_{K(tail)}</td>
<td>*21 ± 4</td>
</tr>
<tr>
<td>I_{Ks(tail)}</td>
<td>7 ± 5</td>
</tr>
</tbody>
</table>

* Statistically significant (p < 0.05).

---

**Fig. 4.** Effect of chloroquine on I_{Ca-L} in cat ventricular myocytes. From a holding potential of −70 mV, test pulses to membrane potential ranging from −30 to +50 mV were applied at a frequency of 0.1 Hz. A, current traces elicited by pulses to −20, −10, 0, and +10 mV, obtained under control conditions and in the presence of chloroquine 10 μM. B, I-V relationships for the peak current amplitude, under control conditions and in the presence of chloroquine, mean ± S.E. of n = 5 cells are shown.

**Fig. 5.** Effect of chloroquine on I_{K1} in cat ventricular myocytes. From a holding potential of −60 mV, test pulses to membrane potentials ranging from −100 to −40 mV were applied at a frequency of 0.1 Hz were applied. A, current traces elicited by test pulses to −100, −90, −80, −60, and −40 mV, obtained under control conditions and in the presence of chloroquine 3 μM. B, I-V relationships for the current measured at the end of the pulses, under control conditions and in the presence of chloroquine, mean ± S.E. of n = 7 cells are shown.
concentrations used were partially reversible on washout, about 60% after 1 and 3 μM and about 40% after 10 μM (data not shown). Possible use-dependent block of I_{Ca-L} by chloroquine was studied using a 1-Hz train of 200-ms pulses to +10 mV, applied from a holding potential of −70 mV. No additional significant use-dependent effects were observed (data not shown).

Effects of Chloroquine on Potassium Currents. I_{K1} was elicited with hyperpolarizing and depolarizing pulses of 500-ms duration to membrane potentials ranging from −100 to −40 mV from a holding potential of −60 mV. Figure 5A shows current traces obtained under control conditions and in the presence of 3 μM chloroquine. The effect of chloroquine on I_{K1} measured at the end of the pulses is shown in Fig. 5B. Chloroquine at 3 μM significantly decreased current amplitude in a voltage-dependent manner. I_{K1}, measured at −40 mV was decreased by 94%, at −60 mV by 86%, and at −100 mV by 67%. In addition, during hyperpolarizing pulses to membrane potentials of −90 and −100 mV, a time-dependent unblock was observed (Fig. 5A, bottom). Table 2 shows the concentration-dependent block by chloroquine. The effects of chloroquine on I_{K1} at all concentrations used were completely reversible on washout (data not shown).

Figure 6 shows the effect of the maximum concentration of chloroquine used in the present work (10 μM) on the transient outward potassium current, I_{to}. I_{to} was elicited by applying depolarizing pulses to membrane potentials ranging from −30 to +50 mV from a holding potential of −60 mV (Fig. 6A). Chloroquine did not significantly affect the peak current amplitude or time course of I_{to}. Peak current-voltage relationships are shown in Fig. 6B. Possible use-dependent effects on I_{to} were studied by applying 2-Hz trains of 200-ms pulses to +50 mV from a holding potential of −60 mV. Chloroquine did not change I_{to} peak amplitude during the train of pulses (data not shown).

In cat ventricular myocytes, as in other mammalian species, I_{K} is composed of the rapid and slow delayed rectifying outward currents, I_{Kr} and I_{KS} (Sanguinetti and Jurkiewicz, 1990; Sanchez-Chapula, 1996; Barajas-Martinez et al., 2000). I_{K} was elicited with 3-s pulses to potentials ranging from −30 to +50 mV, applied from a holding potential of −40 mV. Tail currents were recorded upon repolarization to −40 mV (Fig. 7A). Chloroquine at 3 μM decreased the holding current and the instantaneous current elicited by pulses to −30 and −10 mV, an effect likely caused by blockade of I_{K1} or nondeactivated I_{Kr}. In addition, chloroquine produced a decrease in the amplitude of the time-dependent current, more pronounced at membrane potentials negative to +20 mV (Fig. 7B), and decreased tail current amplitude (Fig. 7C). In the presence of the drug, the I-V relationship of the time-dependent current showed less inward rectification than control. The effects of chloroquine were 95% reversible after washout (data not shown).

Figure 8 shows current traces (Fig. 8A) and I-V relationships of the chloroquine (3 μM)-sensitive time-dependent current amplitude (Fig. 8B), and tail current amplitude (Fig. 8C). The chloroquine-sensitive current showed characteristics similar to I_{Ks}. The slope conductance of the time-dependent I-V relation was negative at potentials positive to 0 mV, and tail current had a threshold potential of −30 mV and reached saturation at +20 mV. The activation curve had a V_{1/2} of −9.5 ± 1.2 mV and a slope factor of 7.1 ± 0.9 mV. These values are similar to those found for the doxifluridine-sensitive current in cat ventricular myocytes (Barajas-Martinez et al., 2000).

MK-499 is a class III antiarrhythmic drug that selectively blocks I_{Kr} (Lynch et al., 1994). Therefore, in the presence of 3 μM MK-499, I_{K} is solely composed by I_{Ks}. In Fig. 9, the effect of chloroquine on the MK-499-resistant current is shown. In the presence of MK-499 alone, the time-dependent current activated during the depolarizing pulses showed a close to linear I-V relationship, and tail current amplitude did not reach saturation at +50 mV. Chloroquine (10 μM) produced a small decrease (11%) in time-dependent (Fig. 9B) and tail current (Fig. 9C) amplitudes. However, this small effect was concentration-independent (Table 2). In addition, these effects were not reversible on washout (data not shown).

One possible limitation of this study could be rundown of ionic currents. However, the effects of chloroquine on I_{Na}, I_{K1},
and IKr were almost completely reversible, and the effect of the drug on ICa-L was partially reversible, making it unlikely that rundown of these currents could explain the decrease in current amplitude observed in the presence of the drug. The effect of chloroquine on IKs was not reversible, in addition, the effects of chloroquine were concentration-independent. These results suggest that IKs rundown may explain the small decrease in IKs measured in the presence of the drug.

**Discussion**

We found that chloroquine (0.3–10 μM) induced a decrease of Vmax, prolonged APD, and decreased maximum diastolic potential in cat isolated Purkinje fibers and ventricular myocytes. Chloroquine also increased the firing frequency of spontaneous activity in cat Purkinje fibers. In addition, after 60 min of superfusion with a concentration 10 μM, in four of five cat Purkinje fibers spontaneous firing was abolished. In experiments under voltage-clamp conditions, chloroquine inhibited I_K1 > I_Kr > I_Na > I_Ca,L. The transient outward potassium current, Ito, and the slow component of IK were not modified by chloroquine. The blocking effects of chloroquine on I_K1 were significantly voltage-dependent, the block increased with depolarization and decreased with hyperpolarization. This profile of voltage dependence is consistent with a positively charged molecule blocking the channel from the intracellular side and entering the pore to such an extent as to be subjected to the transmembrane electrical field (Sniders et al., 1992; Benavides-Haro and Sanchez-Chapula, 2000).

Chloroquine administered at therapeutic concentrations reaches plasma concentrations of 0.29 to 0.48 μM (Webster, 1992). In a retrospective study of patients with acute chloroquine overdose, the mean amount ingested by 167 patients was 4.5 ± 2.8 g. The mean blood chloroquine concentration on admission was 20.5 ± 13.4 μM (Clemessy et al., 1996). Rieu et al. (1988) found in severe chloroquine poisoning, blood levels ranging from 40 to 80 μM. Therefore, the concentrations used in the present study are clinically relevant. Chloroquine, administered at therapeutic concentrations, has been found to produce different cardiovascular effects such as fall in blood pressure, slowing of ventricular conduction, and electrocardiographic changes such as lengthening of QRS and QT intervals (Bustos et al., 1994). Acute poisoning with chloroquine has been reported to produce cardiac failure, rhythm disturbances, atrioventricular block (Guedira et al., 1994), impaired intraventricular conduction, and cardiac arrest (Taboulet and Bismuth, 1994).

To investigate the possible cellular electrophysiological mechanisms responsible of the slowing in ventricular conduction, electrocardiographic changes, and rhythm disturbances, the effects of chloroquine on action potential and the main ionic currents underlying the ventricular action potential were studied. Using microelectrode techniques in multicellular preparations, it was reported that chloroquine depresses the action potential Vmax, suggesting that the drug inhibits INa (Harris et al., 1988). In the present work, we confirmed this assumption by showing that chloroquine at concentrations of 1 μM and higher inhibited INa. Class I antiarrhythmic drugs can be proarrhythmic due to facilitation of re-entrant arrhythmias caused by excessive slowing of
we found that chloroquine at concentrations of 1 μM and vascular resistance (Sofola, 1980). In the present work, dogs produces significant reductions in cardiac contractility and diastolic pressure (Olatunde, 1970). Chloroquine in the basis of the observation that systolic blood pressure fell an effect attributed to depression of cardiac contractility on channels.

In addition, the impaired intraventricular conduction and the excessive lengthening in QT interval as a possible mechanism responsible for the proarrhythmic effects of chloroquine, the increase in automaticity induced by the drug can also be a potential cause of arrhythmias. The decrease in diastolic membrane potential and increase in automaticity induced by chloroquine can be explained by its blocking effects on I_{K1}. During phase 4 of the Purkinje fibers action potential, slow depolarization results from activation of the pacemaker current (I_p) (DiFrancesco and Noble, 1985). Because the maximum diastolic potential is near −90 mV, the decay of the delayed rectifying outward current contributes only a very small current during phase 4. On the other hand, I_{K1} carries a very significant outward current during the pacemaker depolarization, which largely balances the inward current carried by I_p channels. For this reason, factors that change I_{K1} have a large effect on Purkinje fiber rhythm and the maximum diastolic potential (Noble, 1995). However, a direct effect of chloroquine on I_p or electrogenic exchangers and pumps such as Na⁺/K⁺ pump cannot be discounted.

The most frequent cardiovascular manifestations during chloroquine treatment are the electrocardiographic changes, diminution of the T wave, and prolongation of the QTc interval (Bustos et al., 1994; Bouree, 1997). These effects can be explained by the action potential duration increase induced by the drug. Clinical and animal data support the hypothesis that acquired forms of long QT syndrome result from prolonged repolarization that leads to early afterdepolarizations and triggered arrhythmias (Surawicz, 1989). Early afterdepolarizations can be induced by block of potassium currents such as I_{K1} or I_{Kr} (Kaseda et al., 1989), by activation of L-type Ca²⁺ current (January and Riddle, 1989), or inhibition of Na⁺ current inactivation (Boutjdir and El-Sherif, 1991). The most common mechanism of drug-induced torsades de pointes is I_{Kr} inhibition. In our experiments, chloroquine did not induce early afterdepolarizations; however, under voltage-clamp conditions, the most prominent effect of chloroquine on cardiac membrane currents was a marked reduction of I_{K1} and I_{Kr}. The marked reduction of both I_{K1} and I_{Kr} can explain the prolongation of action potential duration induced by the drug (Harris et al., 1988). On the other hand, the reduction of I_{Na} and I_{Ca-L} induced by chloroquine may limit the prolongation of action potential duration and the appearance of afterdepolarizations.

In conclusion, we have found that the antimalarial drug chloroquine at clinically relevant concentrations inhibited several currents in cat ventricular myocytes. These effects can explain most of the electrophysiological modifications and proarrhythmic effects reported for chloroquine in mammalian cardiac preparations.

Acknowledgments

We thank Dr. Michael Sanguinetti for critical reading of the manuscript, and Olivia Mercado Ruiz for technical assistance and preparation of the figures.
Fig. 9. Effects of chloroquine on $I_{\text{Na}}$ of cat ventricular myocytes. From a holding potential of $-40$ mV, test pulses to membrane potentials ranging from $-30$ to $+50$ mV were applied at a frequency of 0.05 Hz, tail current were recorded upon repolarization to the holding potential of $-40$ mV. A, current traces elicited by test pulses to $-30$, $+10$, $+10$, and $+50$ mV, in the presence of MK-489 5 $\mu$M and in the presence of MK-499 3 $\mu$M + chloroquine 10 $\mu$M. B, I-V relationships for the time-dependent current activated during the test depolarizing pulses, in the presence of MK-489 alone and in the presence of MK-489 plus chloroquine, mean $\pm$ S.E. of $n = 4$ cells are shown. C, I-V relationships for the tail current amplitude, in the presence of MK-489 alone and in the presence of MK-489 plus chloroquine, mean $\pm$ S.E. of $n = 4$ cells are shown.

References


Boutjdir M and El-Sherif N (1991) Pharmacological evaluation of early afterdepolarizations elicited by test pulses to 30 mV were applied at a frequency of 0.05 Hz, tail current were recorded upon repolarization to the holding potential of 20 mV. A, current traces elicited by test pulses to -30, +10, +10, and +50 mV, in the presence of MK-489 5 $\mu$M and in the presence of MK-499 3 $\mu$M + chloroquine 10 $\mu$M. B, I-V relationships for the time-dependent current activated during the test depolarizing pulses, in the presence of MK-489 alone and in the presence of MK-489 plus chloroquine, mean $\pm$ S.E. of $n = 4$ cells are shown. C, I-V relationships for the tail current amplitude, in the presence of MK-489 alone and in the presence of MK-489 plus chloroquine, mean $\pm$ S.E. of $n = 4$ cells are shown.


Send reprint requests to: José A. Sánchez-Chapula, M.D., Ph.D., CUIB, Universidad de Colima, Apdo. Postal 199, C.P. 28000, Colima, Col. México.

E-mail: sancheza@cxic.ucol.mx