Effects of Quinidine on Action Potentials and Ionic Currents in Isolated Canine Ventricular Myocytes

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We examined the effects of quinidine (5–20 μM) on transmembrane action potentials and ionic currents of isolated canine ventricular myocytes. Collagenase treatment of canine ventricular tissue produced a yield of 40–60% healthy cells. Myocytes had normal resting and action potentials as measured using conventional microelectrodes. Quinidine decreased \( V_{\text{m}} \), amplitude, overshoot, and the duration of action potentials stimulated by passage of brief current pulses through the recording pipette. Recovery was complete after washout except that action potential duration was prolonged compared with control. A discontinuous single microelectrode voltage (“switch”) clamp was used to measure ionic currents. Quinidine irreversibly reduced steady-state outward current as measured with three different voltage clamp protocols. Quinidine reversibly decreased peak calcium current as well as the slowly inactivating and/or steady-state inward currents in the plateau voltage range, presumably both “late” sodium (tetrodotoxin-sensitive) and calcium (tetrodotoxin-insensitive) currents. The effect on calcium current showed both tonic and use-dependent block. Thus, quinidine has a multitude of actions on both inward and outward currents, which combine to produce the net effect of quinidine on action potential configuration. (Circulation Research 1988;62:324–337)

Quinidine is one of the oldest and most commonly used antiarrhythmic agents and remains among the most effective drugs for control of ventricular arrhythmias. However, the cellular mechanisms by which quinidine exerts its therapeutic effects are not clearly established. Evidence indicates that quinidine inhibits the excitatory sodium current underlying the action potential upstroke.\(^1\) The binding to and blockade of sodium channels is thought to occur preferentially with the open state of the sodium channel, and recovery from block occurs at a relatively slow rate.\(^2\) A voltage- and rate-dependent depression of the maximum rate of depolarization (\( V_{\text{m}} \)) of the action potential by quinidine provides experimental evidence for these hypotheses.\(^3\)

In cardiac Purkinje fibers, quinidine generally increases action potential duration measured during terminal repolarization.\(^4\) This effect has been attributed to a decrease in the delayed outward potassium current, \( I_{\text{k}} \).\(^5\) In other studies, quinidine has been shown to decrease action potential duration,\(^6\) possibly as a result of inhibition of the slowly inactivating and/or steady-state sodium “window” current.\(^7\) Quinidine produces effects on action potential characteristics of ventricular muscle similar to those obtained in Purkinje fibers. Most notably, action potential duration is either decreased or increased, an effect which may be dependent on the concentration of the drug.\(^8\)

Quinidine has also been reported to cause a depression of the action potential plateau and/or an increase in the slope of phase 2.\(^9\) This 'action is associated with a negative inotropic effect of quinidine.\(^9\) The slow inward or calcium current (\( I_{\text{ca}} \)) is an important determinant of the plateau and repolarization phases of the cardiac action potential and of the initiation and maintenance of contraction.\(^10\) The basis for this depressant effect of quinidine on cardiac force may reside in the ability of high concentrations of quinidine to decrease the slow inward current.\(^11\) The aim of the present study was to define the ionic mechanism(s) by which quinidine produces such diverse effects on action potential configuration. We compared the effects of quinidine on the action potential characteristics of intact canine ventricular muscle and isolated canine ventricular myocytes in which the problems associated with external ion accumulation and depletion are minimized. Membrane ionic currents were measured directly in isolated ventricular myocytes using the discontinuous single microelectrode voltage clamp technique.\(^12\) We examined the effects of quinidine on the slowly inactivating sodium current, the slow inward calcium current, and the outward potassium current.

Materials and Methods

Solutions and Drugs

Normal Tyrode's solution (mM): NaCl 135, KCl 5.4, CaCl₂ 1.8, MgCl₂ 1.05, dextrose 5, NaHCO₃, 22.
NaH₂PO₄ 2.4, pH 7.3, gassed with 5% CO₂-95% O₂. 
HEPES-Tyrode's solution (mM): NaCl 142, KCl 5.4, CaCl₂ 1.8, MgCl₂ 0.5, dextrose 5, HEPES 5, adjusted with NaOH to pH 7.4.

Calcium-free cardioplegia solution (mM): NaCl 10, KCl 60, MgCl₂ 5, Na-pyruvate 5, taurine 20, dextrose 20, succrose 100, EGTA 0.5, HEPES 5, adjusted with HCl to pH 7.3.

Enzymatic digestion medium: NaCl 140 mM, KCl 5.4 mM, MgCl₂ 1.5 mM, dextrose 5 mM, HEPES 5 mM, collagenase 0.25% (2.5 mg/ml, Worthington Type II, Freehold, New Jersey), albumin 0.2% (2 mg/ml, bovine albumin, Sigma, St. Louis, Missouri), adjusted with NaOH to pH 7.2.

KB medium: KCl 30 mM, KH₂PO₄ 10 mM, MgCl₂ 5 mM, K₃ATP 5 mM, taurine 15 mM, glutamic acid 70 mM, EGTA 0.5 mM, KOH 5.2 g/l, adjusted with KOH to pH 6.9.

Tetrodotoxin (TTX) and quinidine sulfate were obtained from Sigma. Stock solutions of both drugs were prepared at a concentration of 1 mM and appropriate amounts of the stock solutions were added to the HEPES-Tyrode's solution immediately prior to use.

Procedures for Intact Ventricular Muscle and Isolation of Single Myocytes

Adult mongrel dogs were anesthetized with sodium pentobarbital (40 mg/kg i.v.). The heart was quickly removed and rinsed in cold normal Tyrode's solution. Papillary muscles and trabeculae were dissected from the right ventricle and placed in cold oxygenated normal Tyrode's solution. These preparations were used in the studies of "intact" ventricular myocardium. A portion of myocardium was enzymatically dissociated using a procedure modified from that of Isenberg and Klockner.21 A 2-3-cm block of apical myocardium was obtained by making a transverse incision through the apex of the heart. This ventricular tissue was dissected further into 1-mm³ blocks, then placed in the apex of the heart. This ventricular tissue was obtained by making a transverse incision through the apex of the heart. This ventricular tissue was placed in cold normal Tyrode's solution at a constant rate of 1 Hz via a bipolar extracellular platinum-iridium electrode, which was insulated except at the tip. The electrodes were connected to a constant current source (model 850A, WPI, New Haven, Connecticut) which was triggered by a digital interval generator (model 830, WPI). Stimuli were depolarizing square wave pulses at 1 msec in duration with an intensity of 1.5 times threshold. Glass microelectrodes filled with 3 M KCl and which had tip resistances of 10–30 MΩ were used to record transmembrane potential. The microelectrode was connected through an Ag-AgCl junction to the input of a high impedance capacitance-neutralizing amplifier (Picosystems 181, Instrumentation Laboratories, Boston, Massachusetts). Continuous single impalements were maintained throughout all experiments. A 30-minute control period, during which no alterations of action potential parameters were noted, preceded the initiation of each experiment.

Isolated ventricular myocyte studies. The isolated ventricular myocytes were transferred to a glass bottom experimental chamber (12 × 25 mm) filled with HEPES-Tyrode's solution to a height of 2 mm. After the myocytes settled and attached to the bottom of the chamber, they were continuously superfused with preheated HEPES-Tyrode's solution with a pH of 7.4 at a rate of 1.5 ml/min. The temperature was monitored and maintained at 37 °C. The experimental chamber was placed on the stage of an inverted microscope (Nikon Diaphot-TMD). The cells could be viewed either through the binocular eyepieces or on a closed-circuit video monitor (model FVM 95A, NEC) via a video camera (model TC2000, RCA) attached to the side camera port of the microscope. Transmembrane potentials were recorded by means of conventional glass microelectrodes that were filled with 3 M KCl and had tip resistances of 35–55 MΩ. The microelectrode was connected through an Ag-AgCl electrode to the headstage preamplifier (model HS-2L, gain = 0.1) of an operational amplifier with high input impedance and variable input capacitance neutralization (Axoclamp-2, Axon Instruments, Burlingame, California). The voltage drop across the microelectrode produced by current injection was compensated electronically with an active bridge circuit immediately before impalement of a cell. The experimental bath was grounded through a 3 M KCl agar bridge and Ag-AgCl junction. The microelectrodes were positioned with Huxley micromanipulators at the cell surface and impalement of the cell was achieved by briefly (1 msec) increasing the input capacitance neutralization to cause oscillation.

After establishing a stable microelectrode seal, the myocyte was lifted off the bottom of the chamber to allow complete superfusion of the cell. Action potentials were generated by passing current pulses of 1 msec in duration, and with an intensity of 1.2 times threshold, through the recording electrode.

General electrophysiological procedures. Voltage and current signals were displayed on a storage oscilloscope (model 5111A, Tektronix) and were monitored and maintained at 37 ± 0.5°C. The muscles were stimulated at a constant rate of 1 Hz via a bipolar extracellular platinum-iridium electrode, which was insulated except at the tip. The electrodes were connected to a constant current source (model 850A, WPI, New Haven, Connecticut) which was triggered by a digital interval generator (model 830, WPI). Stimuli were depolarizing square wave pulses at 1 msec in duration with an intensity of 1.5 times threshold. Glass microelectrodes filled with 3 M KCl and which had tip resistances of 10–30 MΩ were used to record transmembrane potential. The microelectrode was connected through a Ag-AgCl junction to the input of a high impedance capacitance-neutralizing amplifier (Picosystems 181, Instrumentation Laboratories, Boston, Massachusetts). Continuous single impalements were maintained throughout all experiments. A 30-minute control period, during which no alterations of action potential parameters were noted, preceded the initiation of each experiment.
photographed intermittently during the experiment or were recorded on a strip chart recorder (model 220, Gould, Cleveland, Ohio). The voltage and current signals also were analyzed by a Norland 3001 digital waveform analysis system (sampling frequency, 2 kHz) that performed rapid on-line data measurement of the following: transmembrane maximum diastolic potential (MDP), action potential amplitude (APA), action potential overshoot (OS), action potential duration at 50% (APD_{50}) and 90% (APD_{90}) of repolarization, and the maximum rate of depolarization (dV/dt) of phase 0 (V_{m0}). The transmembrane voltage signal was input to an electronic differentiator that had a linear response in the range of 100–1,000 V/sec to determine V_{m0} of the action potential. For each experimental condition, 5 or 10 measurements of the above parameters were averaged.

Voltage Clamp Experiments

Voltage clamp experiments were performed with the Axoclamp-2 in the discontinuous single-electrode voltage clamp (dSEVC) or “switch” clamp mode. In this mode, the tasks of voltage-recording and current-passing are allocated to the same electrode; that is, time-sharing techniques are used to prevent interactions between the two tasks. The cycling rate of the dSEVC was 4–5 kHz and the current signal was filtered with an upper cut-off frequency of 0.3 kHz.

In the first voltage clamp protocol, the steady-state membrane currents were measured using a slowly rising voltage ramp, similar to that used by Colatsky. The membrane potential was held initially at −90 mV, then a 1.5-mV/sec, 80-mV depolarizing ramp was applied as the command signal for the voltage clamp (Figure 4). Because the voltage is never held constant (i.e., it is very slowly increasing), this protocol does not provide a true measure of the absolute steady-state currents. Nevertheless, the current-voltage relations obtained with this protocol were virtually indistinguishable from those obtained at the end of 10-second long depolarizing clamp steps. The advantages of the ramp protocol were that it provided a complete current-voltage relation over the range studied, it was obtained rapidly, and it was less damaging to the cells, especially at depolarized potentials. In other voltage clamp protocols, the membrane voltage was clamped at holding potential (V_h), then was step depolarized or hyperpolarized in 10-mV increments to various test potentials (V_c). The duration and frequency of the clamp steps were varied appropriately (see “Results”).

A separate voltage clamp protocol was used to study the effect of quinidine on the “slow inward” or calcium current (I_{ca}). In these experiments, V_h was −40 mV, then was step depolarized or hyperpolarized in 10-mV increments to various test potentials (V_c). The concentration of quinidine was applied, and, when possible, a period after washout of the drug. Sufficient time was allowed to obtain a steady-state condition for drug action. When appropriate, between each experimental protocol, the cell was stimulated at a frequency of 1 Hz to monitor action potential characteristics. We studied the effects of quinidine on action potential characteristics at concentrations of 1.6 and 3.2 μg/ml (5 and 10 μM), which is within the range of clinically useful plasma concentrations. Because of the small amplitude of the currents (<2 nA) measured and because quinidine at concentrations of 3.2 μg/ml (10 μM) or less often produced small changes in ionic currents (e.g., slowly inactivating sodium and calcium currents), we chose to use a standard quinidine concentration of 6.5 μg/ml (20 μM) in most of the voltage clamp experiments. This concentration was intermediate as compared with that used in other voltage clamp studies (2–50 μg/ml).²⁰

Advantages and Limitations of dSEVC Method

An essential goal of these experiments was to maintain the isolated myocytes in as normal a physiological state as possible for as long as possible in order to accurately assess the effect of the drug. Therefore, to avoid disruption of the intracellular biochemical and ionic environments, we chose to use the dSEVC method for monitoring transmembrane potential and ionic currents. Using this technique, myocytes remained stable for hours. Action potential characteristics and membrane currents were unchanged during 30-minute control periods and returned to control values after exposure to drugs that washed out completely. The stability of I_{ca} was crucial since rundown of this current is common when using a suction pipette technique.²⁷

One concern regarding the method of dSEVC or “switch” clamp is the adequacy of voltage control using a single microelectrode for both voltage measurements and current passage. Uniform voltage control was confirmed in three experiments with an independent microelectrode at a distant site in the cell. Figure 1 shows the voltages measured by the “switch” clamp microelectrode (V_h, top trace), and by an independent microelectrode (V_m, middle trace). Voltage-clamp steps 100 msec in duration were applied from a V_h of −75 mV to a V_h of 0 mV. At a V_h of −75 mV, both microelectrodes recorded the same voltage while passing −0.75 nA of current, indicating that during
Quinidine Effects on Canine Ventricular Myocytes

Salata and Wasserstrom

Results

Action Potential Studies

Intact ventricular muscle. Figure 2 demonstrates the effect of 10 μM quinidine on the action potential configuration recorded from a dog papillary muscle. Three superimposed recordings are shown. Quinidine decreased the overshoot and the amplitude of the action potential without affecting the maximum diastolic potential. Quinidine also reduced plateau height, and shortened the action potential duration in the plateau range but prolonged terminal repolarization, and thereby producing a crossover of the control action potential. Plateau height and duration recovered after washout of quinidine. In addition, APD₉₀ increased beyond the control value during the washout period. Quantitative data and statistical analysis of the effects of quinidine at concentrations of 5 and 10 μM are presented in Table 2. Quinidine at both concentrations significantly decreased the amplitude, overshoot, and V₉₀ of the action potential but had no significant effect on the maximum diastolic potential. In contrast to the effect on papillary muscles, quinidine significantly decreased APD₉₀ and APD₆₀. The effects of quinidine on the action potential were reversed after washout of the drug. However, the action potential was prolonged beyond the control duration in 7 of 12 experiments for which some period of washout was obtained, but overall, the effect was not statistically significant. When observed, the prolongation was maintained throughout the recorded washout period of at least 15 minutes and as long as 45 minutes (Figures 3 and 4). This effect was greater after 10 μM quinidine.

Effect on Steady-State Membrane Currents

The effects of quinidine (20 μM) on the steady-state membrane currents were assessed in 10 experiments using the ramp clamp protocol. The results of a typical experiment are illustrated in Figures 4 and 5. The control current-voltage relation has a characteristic N shape with a region of inward rectification and negative slope and is similar to that obtained in intact ventricular muscles.

Figure 1. Adequacy of voltage control using the single microelectrode switch clamp. The top trace shows the voltage recorded by the switch clamp microelectrode positioned in the center of the ventricular myocytes (Vⱼ). The middle trace shows the voltage recorded by an independent microelectrode positioned near the end of the cell at an interelectrode distance of 50 micrometers (Vᵢ). The Vⱼ trace is filtered at an upper cut-off frequency of 100 Hz to eliminate noise generated by the switching amplifier, which cycled at 4.5 kHz. The bottom trace shows the current measured using the switch clamp technique.

Figure 2. Effect of quinidine on the action potential configuration of a canine papillary muscle. After a stable control (C) period of 30 minutes, the preparation was exposed to 10 μM quinidine (Q) for 20 minutes, followed by a 20-minute washout (W) period. Tracings are superimposed digitized records obtained at a stimulation frequency of 1 Hz. V₉₀ was 186 V/sec during the control and was reduced to 86 V/sec after quinidine (not shown). Preparation No. 250386-3.
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Period of 10 minutes, the cell was exposed to quinidine (5 nM) for 10 minutes (center panel). The right panel was obtained after 15 minutes of washout of the drug. Note the return to control of \( V_{\text{m}} \) and the prolongation of action potential duration during the washout. Cell No. 031285-1.

Table 1. Effects of Quinidine on Action Potential Characteristics of Intact Dog Ventricular Muscle

<table>
<thead>
<tr>
<th></th>
<th>MDP (mV)</th>
<th>OS (mV)</th>
<th>AMP (mV)</th>
<th>APD_{90} (msec)</th>
<th>APD_{99} (msec)</th>
<th>( V_{\text{m}} ) (V/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-74.2 ± 2.5</td>
<td>21.3 ± 2.1</td>
<td>95.4 ± 2.1</td>
<td>132.6 ± 7.9</td>
<td>163.2 ± 10.8</td>
<td>236.4 ± 49.1</td>
</tr>
<tr>
<td>Quinidine (5 µM)</td>
<td>-75.9 ± 2.6</td>
<td>20.7 ± 1.6</td>
<td>96.6 ± 1.9</td>
<td>139.4 ± 7.1*</td>
<td>173.0 ± 9.5*</td>
<td>207.6 ± 46.9*</td>
</tr>
<tr>
<td>Control</td>
<td>-74.4 ± 2.1</td>
<td>19.6 ± 2.4</td>
<td>94.0 ± 2.2</td>
<td>127.3 ± 8.5</td>
<td>175.5 ± 17.0</td>
<td>230.7 ± 40.5</td>
</tr>
<tr>
<td>Quinidine (10 µM)</td>
<td>-75.0 ± 1.9</td>
<td>18.3 ± 1.8</td>
<td>93.3 ± 2.6</td>
<td>137.0 ± 8.2†</td>
<td>202.2 ± 24.6†</td>
<td>156.1 ± 37.5†</td>
</tr>
<tr>
<td>Control</td>
<td>-75.8 ± 2.5</td>
<td>19.8 ± 1.9</td>
<td>95.6 ± 2.5</td>
<td>132.2 ± 9.6</td>
<td>163.2 ± 13.4</td>
<td>236.4 ± 49.1</td>
</tr>
<tr>
<td>Washout</td>
<td>-77.0 ± 1.9</td>
<td>20.4 ± 2.1</td>
<td>97.3 ± 3.8</td>
<td>155.9 ± 9.1†</td>
<td>192.8 ± 11.5†</td>
<td>220.8 ± 69.4†</td>
</tr>
</tbody>
</table>

Values are presented as the mean ± SEM and were compared using Student's paired t-test. Individual observations are the average of 10 action potentials at a stimulation frequency of 1 Hz. MDP, maximum diastolic potential; OS, overshoot; AMP, amplitude; APD_{90} and APD_{99}, action potential duration at 50% and 90% of repolarization, respectively; \( V_{\text{m}} \), maximum upstroke velocity (dV/dt) of phase 0 of action potential. Numbers in parentheses equal the number of experiments. Washout values are after exposure to 10 µM quinidine.

\(* p<0.05 \) and \( \dagger p<0.01 \) compared with control.

Effect of quinidine on action potential configuration and \( V_{\text{m}} \) of an isolated dog ventricular myocyte. Each panel shows 3 superimposed action potentials at a stimulation frequency of 1 Hz, emphasizing the stability of the recordings. After a stable control period of 10 minutes, the cell was exposed to quinidine (5 µM) for 10 minutes (center panel). The right panel was obtained after 15 minutes of washout of the drug. Note the return to control of \( V_{\text{m}} \) and the prolongation of action potential duration during the washout. Cell No. 031285-1.

\[ dV/dt \] of phase 0 of action potential.
Table 2. Effects of Quinidine on Action Potential Characteristics of Isolated Dog Ventricular Myocytes

<table>
<thead>
<tr>
<th>Condition</th>
<th>MDP (mV)</th>
<th>OS (mV)</th>
<th>AMP (mV)</th>
<th>APD50 (msec)</th>
<th>APD90 (msec)</th>
<th>V_{max} (V/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-76.5 ± 1.0 (11)</td>
<td>27.0 ± 2.1 (11)</td>
<td>103.5 ± 2.5 (11)</td>
<td>102.4 ± 19.1 (11)</td>
<td>140.5 ± 21.5 (11)</td>
<td>317.3 ± 17.5 (11)</td>
</tr>
<tr>
<td>Quinidine (5μM)</td>
<td>-76.0 ± 1.0 (11)</td>
<td>18.2 ± 3.2 (11)</td>
<td>94.9 ± 3.7 (11)</td>
<td>82.3 ± 11.0 (11)*</td>
<td>112.7 ± 13.1 (11)*</td>
<td>223.2 ± 19.5 (11)*</td>
</tr>
<tr>
<td>Washout</td>
<td>-75.2 ± 1.0 (6)</td>
<td>27.0 ± 3.5 (6)</td>
<td>101.8 ± 4.3 (6)</td>
<td>98.7 ± 22.0 (6)</td>
<td>144.2 ± 25.2 (6)</td>
<td>306.7 ± 35.7 (6)</td>
</tr>
<tr>
<td>Control</td>
<td>-74.3 ± 1.6 (7)</td>
<td>31.4 ± 2.6 (7)</td>
<td>108.9 ± 4.5 (7)</td>
<td>129.5 ± 22.8 (7)</td>
<td>178.3 ± 26.8 (7)</td>
<td>377.5 ± 48.7 (6)</td>
</tr>
<tr>
<td>Quinidine (10μM)</td>
<td>-73.4 ± 1.8 (7)</td>
<td>22.0 ± 3.6 (7)</td>
<td>100.4 ± 5.5 (7)</td>
<td>104.9 ± 16.1 (7)*</td>
<td>147.4 ± 20.9 (7)*</td>
<td>212.5 ± 47.6 (7)*</td>
</tr>
<tr>
<td>Washout</td>
<td>-72.2 ± 1.8 (6)</td>
<td>31.2 ± 3.7 (6)</td>
<td>107.5 ± 6.0 (6)</td>
<td>139.3 ± 27.1 (6)</td>
<td>190.0 ± 34.2 (6)</td>
<td>337.0 ± 52.0 (5)</td>
</tr>
</tbody>
</table>

MDP, maximum diastolic potential; OS, overshoot; AMP, amplitude; APD_{50} and APD_{90}, action potential duration, at 50% and 90% of repolarization, respectively; \( V_{\text{max}} \), maximum upstroke velocity (dV/dt) of phase 0 of action potential. Numbers in parentheses equal the number of experiments.

Individual observations are the average of 5 action potentials at a stimulation frequency of 1 Hz.

* \( p < 0.05 \), ** \( p < 0.01 \), and *** \( p < 0.001 \) compared with control.

displayed inward rectification consistent with the known properties of \( I_{K1} \). Quinidine decreased \( I_{K1} \) to 0.2 nA and affected the current-voltage relation over this voltage range in a qualitatively identical manner to that obtained using the ramp clamp protocol in Figure 5. The effect on \( I_{K1} \) was not reversible after washout of quinidine for periods of up to 45 minutes. The same effects were observed in two additional experiments.

Quinidine affects other ionic currents, for instance both inward sodium and calcium currents (see below), in a use- and frequency-dependent manner. Therefore, we tested whether or not the effect of quinidine on \( I_{K1} \), displayed use- or frequency-dependence in three additional experiments. Once a complete current-voltage relation was obtained as in Figure 6A, we applied trains of 10 hyperpolarizing clamp steps to a given test potential (usually \(-100 \text{ mV}\)) at various frequencies (0.1 to 1 Hz). Under these conditions, quinidine decreased \( I_{K1} \) to the same degree for a given test potential regardless of frequency or position or step number in the train. The effects for hyperpolarizing pulses were also independent of the holding potential, but quinidine inhibited the current in a dose-dependent manner (cf. top panel of Figure 6B). A similar dose-dependent inhibition of \( I_{K1} \), occurred with depolarizing clamp steps (bottom panel of Figure 6B), but was also use- and frequency-independent.

**Effect on Slowly Inactivating or Steady-State ("Window") Sodium Current**

During the cardiac action potential, the flow of sodium (Na\(^+\)) current is not restricted to the initial fast phase of depolarization. Cardiac Purkinje cells possess a maintained component of TTX-sensitive Na\(^+\) current, which is a slowly inactivating and/or noninactivating, steady-state "window" current.\(^5,13,14\) We have demonstrated that this Na\(^+\) current contributes substantially to maintaining the plateau and prolonging action potential duration in these isolated ventricular myocytes (J.A. Wasserstrom and J.J. Salata, manuscript in review).

We tested the hypothesis that quinidine reduces the slowly inactivating Na\(^+\) current in 6 experiments. The \( V_h \) was usually \(-90 \text{ mV}\) and depolarizing steps of 5 seconds in duration were applied to various \( V_h \) in 10-mV increments at a frequency of 0.05 Hz. Membrane currents were measured at various times (0.1, 0.25, 0.5, 1, and 5 seconds) during the voltage clamp step. The effect of quinidine or TTX alone or a combination of both drugs was studied to determine if quinidine...
affects the TTX-sensitive Na⁺ current during the plateau of the action potential. Figure 7A shows the effects of TTX alone and in combination with quinidine during depolarizing voltage clamp steps from —90 mV to —60 mV and —30 mV. TTX alone caused an outward shift in the current at both representative Vt, especially at earlier times in the clamp steps, thereby eliminating the time-dependent outward shift in the current. Addition of quinidine decreased the holding current at —90 mV, shifted the current inward at —60 mV, and produced a further outward shift at —30 mV. The current-voltage relation for this experiment is plotted in Figure 7B. TTX alone shifted the current-voltage relation in the outward direction in a manner similar to that observed previously by us (J.A. Was- serstrom and J.J. Salata, manuscript in review) and others. The amplitude of the TTX-induced current shift was greater at earlier times (<1 second) and was smaller at later times in the clamp step. This result indicates that TTX blocked a time-dependent inward current and confirms the presence of a TTX-sensitive Na⁺ current during the plateau of the action potential in these ventricular myocytes.

Addition of quinidine produced an inward shift in the current-voltage relation at potentials between —80 and —40 mV and an outward shift at potentials more negative than the resting potential of —83 mV (cf. change in holding current at —90 mV in top panel). These results were the same as those observed in the absence of TTX as described earlier. Quinidine produced a further outward shift beyond that produced by TTX at —30 mV and less negative potentials. At potentials less negative than —40 mV (i.e., in the plateau voltage range) the effects of quinidine on the

**Figure 5.** Effect of quinidine on the steady-state current-voltage relation for an isolated dog ventricular myocyte. Superimposed curves were obtained during control, after 25 minutes of exposure to quinidine (20 μM), and after 25 minutes of washout of the drug. Data are digitized values from the experiment of Figure 4 using the ramp clamp protocol. Cell No. 060286-3.

**Figure 6.** Effect of quinidine on the time-independent background K⁺ conductance or inward rectifier, I_k. Panel A shows the current-voltage relation obtained using the voltage clamp protocol depicted in the inset. Steady-state current (I) was measured at the end of the 500-msec clamp step. In this example, clamp steps were applied at a frequency of 0.5 Hz, but results obtained at other frequencies (0.1 to 1 Hz) were superimposable, indicating a lack of use- or frequency-dependence, even with duty cycles at 1 Hz. Panel B confirms the lack of use- and frequency-dependence using both hyperpolarizing and depolarizing clamp steps (inset), although there was a dose-dependent inhibition of I_k at each test potential. The data were identical for the first and last test pulse in a train of 10 pulses at all frequencies tested. A: Cell No. 090986-1; B: Cell No. 231086-2.
current-voltage relation relative to control were nearly identical in both the presence and absence of TTX (Figures 5 and 7). However, at less negative test potentials (> −30 mV), the amplitude of the outward current shift (i.e., the absolute change in current produced by quinidine in the presence of TTX) even at higher TTX concentrations (40 μM), was less than with quinidine alone. The quinidine-induced outward shift in the current-voltage relation at these potentials reversed after washout of the drug both in the absence and presence of TTX. Similar results were obtained in all six experiments of this type. These results indicate that part of the outward shift produced by quinidine at less negative membrane potentials was due to a reversible inhibition of the TTX-sensitive Na+ current. However, it appears that quinidine also reversibly affected another inward current, possibly the slow inward or calcium current.

Effect on Slow Inward or Calcium Current (I_{Ca})

The hypothesis that quinidine reduced I_{Ca} was examined in a series of 8 experiments. Figure 8 shows an example of the effect of quinidine on the peak I_{Ca}. Quinidine clearly reduced the peak I_{Ca} at all test potentials. A plot of the current-voltage relation for the peak I_{Ca} is presented in Figure 9, which also illustrates this effect. Quinidine significantly reduced I_{Ca} at test potentials between −10 and +30 mV. Notably, the effect of quinidine on I_{Ca} reversed after washout of the drug, indicating that the effect of quinidine could not be attributed to rundown of I_{Ca}.

For comparison, in 3 experiments after the washout of quinidine, we assessed the effect of verapamil (5 μM), which produced a much greater inhibition of I_{Ca}. Additionally, in a few experiments, higher concentrations of verapamil (10 μM) or D600 (5 μM) or CdCl₂ (100 μM) inhibited almost all detectable I_{Ca} after 15 minutes of superfusion (not shown).

Calcium channel antagonists typically exhibit voltage- and use-dependent inhibition of I_{Ca}. We tested whether or not quinidine inhibits I_{Ca} in a use-dependent fashion in another group of experiments. Trains of voltage clamp steps 200 msec in duration were applied from a V_h of −40 mV to a V_m of 0 mV at varying frequencies (0.5 to 3 Hz) and I_{Ca} was monitored. Trains were 30 seconds in duration and a 60-second rest period was interposed between each train. Figure 10 compares the control current tracings with those obtained after 15 minutes of exposure at rest to 20 μM quinidine. During control, the amplitude of the peak I_{Ca} changed biphasically, first increasing slightly to an early maximum, then decreasing slowly thereafter. Both the incline and

**Figure 7.** Effects of tetrodotoxin (TTX) and quinidine on transmembrane currents recorded during 5 second clamp steps from V_h of −90 mV to V_m of −60 and −30 mV. Panel A: Sample tracings are strip chart recordings obtained during control, after 10 minutes of TTX (20 μM) alone, and at 10 minutes after addition of quinidine (20 μM) to the TTX-containing superfuse. Panel B: superimposed current-voltage relations for the experiment of panel A. Plotted currents are digitized values measured at 1 second after initiation of the voltage clamp steps. Cell No. 050286-1.
FIGURE 8. Effect of quinidine on the calcium current of an isolated dog ventricular myocyte. In the bottom panels are the superimposed current traces obtained during 100 msec depolarizing clamp steps from a V, of —40 mV to various V, (top panel). The numbers in the bottom panel refer to the V, and indicate the peak calcium current at the given test potential. Control currents were obtained in the presence of tetrodotoxin (TTX; 10 μM), BaCl₂ (5 mM), and 4-aminopyridine (2 mM). The right panel was obtained at 7 minutes after addition of quinidine (20 μM). Cell No. 200286-6.

FIGURE 9. Effect of quinidine on the current-voltage relation for the peak calcium current. Plotted data are the mean ± SEM (n = 8) peak inward calcium currents (I,Ca) at the various V, during control (10 μM tetrodotoxin, 5 mM BaCl₂, 2 mM 4-aminopyridine), quinidine (20 μM), and after washout of the drug. Comparison data for verapamil (5 μM) were obtained after washout of quinidine in 3 experiments. Peak current amplitudes were measured as the difference between the peak current and the current at the end of the 100-msec clamp step. Statistical significance determined by paired t test.

decline of the peak I, occurs more rapidly at higher frequencies.

In the presence of quinidine, the first peak I, of each train was reduced to the same degree. This tonic block occurred for the very first clamp step when applied after about 10 minutes of exposure at rest to quinidine and persisted even if the rest interval between trains was increased to as long as 3 minutes (not shown). More importantly, progressive reduction of the peak I, occurred during the trains. The rate and degree of inhibition increased dramatically with increasing frequency. These results indicate that the inhibition of the peak I, by quinidine is also use- and frequency-dependent (see also Figure 11).

Calcium current in cardiac cells exhibits a maintained "late" component which may be either slowly inactivating or non-inactivating steady-state I, and which maintains the plateau of the action potential. In Figure 10, the decay or inactivation of I, was incomplete at the end of the 200-msec long clamp steps (cf., control at 1 Hz). The late I, decreased progressively during the control trains, and the decline was faster at higher frequencies. Quinidine inhibited the late component of I, and the use-dependence of this effect appeared to be substantially greater than the effect on the peak I,.

In other words, the envelope of the late I, declined more rapidly than that of the peak I, especially at higher frequencies. The arrows in Figure 10 highlight this effect by pointing to the moment at which the inhibition of the late I, reached the holding current level. Clearly, at higher frequencies this degree of inhibition occurred much earlier.
FIGURE 10. Use- and frequency-dependent effect of quinidine on the calcium current of an isolated dog ventricular myocyte. Tracings are strip chart recordings of the currents measured during the first 15 seconds of 30-second trains of 200-msec long voltage steps from a V1 of −40 mV to a V2 of 0 mV applied at frequencies of 1, 2, and 3 Hz. The solid lines and circles (upper tracings of each panel) outline the envelope of the peak (INa) during control conditions. The dashed lines and squares (lower tracings) are after 15 minutes of 20 μM quinidine. Both tonic and use-dependent block of the peak INa were observed, and the use-dependent block was greater at higher frequencies. Note also that quinidine produced tonic and use-dependent inhibition of the late component of the calcium current, emphasized by the arrows. Cell No. 151086-2.

Figure 11 shows a quantitative assessment of the frequency-dependence of the inhibition of the peak and late ICa by quinidine. The data are standardized by comparing the current amplitudes in the presence of quinidine to those at the same time and condition during the control. Overall, quinidine produced approximately a 15% tonic reduction (first postrest step) and a significant frequency-dependent inhibition of the peak INa (r = 0.85, p<0.01). A much larger tonic inhibition (55%) of the late ICa was observed and, overall, inhibition of this component increased slightly but not significantly with frequency (see “Discussion”).

Discussion

Effects of Quinidine on K+ Currents

Several investigators have recently reported a variety of actions of quinidine on outward K+ currents. Quinidine decreased the time-dependent delayed rectifier (IK1) in both rabbit10 and sheep11 Purkinje fibers, as evidenced by a decrease in the amplitude of the tail currents. Colatsky10 also demonstrated that quinidine caused an inward shift of steady-state plateau currents as well as a decrease in holding current at a holding potential of −43 mV in rabbit Purkinje fibers. This might have indicated an effect on the background K+ conductance, IK1. In contrast, quinidine caused an outward shift of the steady-state current-voltage relation in sheep Purkinje fibers,11 particularly in the plateau voltage range. This latter effect may not be a direct action of quinidine on outward K+ currents, but may result instead from 1) an action of quinidine to block slowly inactivating or steady-state “window” Na+ and/or Ca2+ currents in this voltage range (see below); 2) the anticholinergic action of quinidine that occurs in sheep Purkinje fibers and which is responsible for a shortening of the action potential in this tissue,
an effect that is blocked by atropine (G.I. Sawicki, J.J. Salata, J.A. Wasserstrom, and M.E. Arnsdorf, unpublished observation); and 3) quinidine also blocks the transient outward current (I_o), which is largely carried by K⁺ ions, in canine Purkinje fibers.11

In the present study, although we looked for I_K, we never found any evidence that the current existed in these cells. We were unable to observe tail currents previously defined as resulting from activation of a I_K in the appropriate voltage range (data not shown). This may be the result of the fact that the current tails were too small for our system to resolve. Gintant et al12 commonly measured tail currents with an amplitude of about 300 pA in isolated dog Purkinje cells, which is within the measurable range of our system. Instead, this current may be absent in canine ventricular myocytes.

We did find that quinidine profoundly decreased I_K, an effect that was particularly apparent in voltage ranges negative to —40 mV. This resulted in a decrease in current on either side of the apparent reversal potential, and was more pronounced at very negative potentials (—110 mV), supporting the fact that the current affected by quinidine in this voltage range was the background K⁺ current (I_K). The apparent reversal potential of the current blocked by quinidine is less negative than the estimated value of —85 mV for an extracellular K⁺ concentration of 5.4 mM. This result may be due to the existence of a TTX-sensitive Na⁺ current that is also blocked by quinidine at potentials as negative as —70 mV. The effect of quinidine was qualitatively the same when analyzed with three separate protocols: a voltage ramp and either depolarizing or hyperpolarizing pulses. With the latter two protocols, we demonstrated that the inhibition of I_K by quinidine was independent of voltage, use, and frequency of stimulation. Furthermore, the inhibition was dose-dependent and irreversible even after a period of washout (15–45 minutes) sufficient to allow full recovery of V_m of the action potential and other ionic currents (see below). Therefore, quinidine seems to have its primary direct action on I_K.

Effects of Quinidine on Peak and Slowly Inactivating "Late" I_K Current

The suggestion that a Na⁺ current exists during the plateau of the action potential initially came from the observation that TTX decreased the action potential duration of Purkinje fibers.33 Since then, studies in Purkinje fibers have demonstrated not only a TTX-sensitive, steady-state Na⁺ window current16 but also a slowly decaying component of the Na⁺ current44 that is also inhibited by quinidine and local anesthetic agents.51 A similar TTX-sensitive Na⁺ current is also present in the plateau region of action potentials in these isolated dog ventricular myocytes (J.A. Wasserstrom and J.J. Salata, manuscript in review).

In the present study, we observed that quinidine has a potent reversible blocking action on V_m. Although the relation between V_m and I_K may not be linear,14 V_m fell by nearly 40% in the presence of quinidine (cf. Table 2). This finding strongly suggests that quinidine blocks Na⁺ channels.1 We also showed evidence that quinidine might block the slowly inactivating or "window" Na⁺ current that occurs in the plateau voltage range. Quinidine produced a significant time-dependent outward shift of the current-voltage relation in the plateau voltage range (Figure 7). The degree of this inhibition with quinidine was diminished in the presence of TTX, suggesting that part of the effect of quinidine was TTX-sensitive and carried through Na⁺ channels. We used TTX doses that should have been sufficient to block virtually all Na⁺ channel current. We also showed that part of quinidine’s block of plateau currents was TTX-insensitive. This may represent an effect of quinidine on a slowly inactivating or steady-state "window" Ca²⁺ current as described below. Because of the prominent actions of quinidine on V_m as well as the TTX-sensitive plateau current, we conclude that quinidine indeed blocks Na⁺ channel currents including the slowly inactivating and/or steady-state "window" Na⁺ current. Our conclusion agrees with that of Carmeliet et al who showed also that the block of slowly inactivating Na⁺ current by quinidine is highly use-dependent.5,11

Effects of Quinidine on Peak and Slowly Inactivating "Late" I_Ca

Several investigators have reported that quinidine decreases peak I_Ca in both frog atrium18 and cat papillary muscle.13 This is thought to underlie the negative inotropic actions of quinidine.18,46 Two kinds of Ca²⁺ current (channels) have been described in cardiac cells.18 One is transient or quickly inactivating and activates at a relatively negative membrane potential. Another is long-lasting or slowly inactivating and activates at a more depolarized membrane potential. Evidence indicates that in ventricular cells only the latter type exists,39 and only this current type was examined in the present study. We have demonstrated that quinidine reduces I_Ca in dog ventricular myocytes. We recorded stable control I_Ca for up to 30 minutes before exposure to quinidine and, furthermore, we observed that the inhibitory actions of quinidine on I_Ca reversed nearly to control after washout. Thus, we did not find rundown of I_Ca that is characteristic of similar measurements obtained using the suction pipet technique.27 During control pulse trains, the initial increase in I_Ca after the first depolarizing steps probably reflects the activation of residual I_Ca, which then fails to recover completely from inactivation with frequent repetitive steps. The subsequent gradual decline of I_Ca to a steady state during the control trains occurs more rapidly and to a greater extent at higher frequencies and is most likely due to incomplete recovery from inactivation of I_Ca or modified gating as a result of increased intracellular Ca²⁺. When inhibition of peak I_Ca by quinidine was present, pauses of up to several minutes were not sufficient to eliminate block completely with these voltage clamp conditions. The first pulse following a pause in the train of clamp steps still showed significant block. We interpret this observation to indicate the presence of tonic block of I_Ca by quinidine.
More importantly, use-dependent inhibition of \( I_{Ca} \) by quinidine was revealed at stimulation frequencies of 1 Hz and higher (Figures 10 and 11). Thus, the current-voltage relation of Figure 9, which was obtained at 1 Hz, contains both tonic and use-dependent components, both of which would have been invoked in the effect on the action potential. At higher stimulation frequencies (>1 Hz) the degree of inhibition of peak \( I_{Ca} \) increased and therefore would play a larger role in the effect of quinidine on the cardiac action potential.

Our measurements of peak \( I_{Ca} \) could be slightly distorted in the time to peak current as a result of the delay in the settling time of the dSEVC technique. Therefore, we limited our analysis to the effect of quinidine on the amplitude of the peak \( I_{Ca} \). Any errors arising from the dSEVC technique would tend to reduce the amplitude of the transients such as peak \( I_{Ca} \) and, as a result, the inhibition of \( I_{Ca} \) by quinidine, if anything, could be underestimated.

In addition to blocking peak \( I_{Ca} \), quinidine also reduced a TTX-insensitive late inward current in the plateau voltage range. These experiments indicate that this action of quinidine is most likely due to blockade of a slowly inactivating or steady-state “window” \( Ca^{2+} \) current. The effect of quinidine on this late component of \( I_{Ca} \) appeared to be both a component of tonic block as well as added block with use, especially at higher frequencies (Figure 10). Because this late component of \( I_{Ca} \) was usually very small (<0.1 nA) and difficult to resolve accurately, Figure 11 may be an underestimate of the frequency-dependence of the inhibition of the late \( I_{Ca} \). Furthermore, the data of Figure 11 were standardized at 15 seconds after initiation of the train, at which time the frequency-dependence of the effect may have reached a steady level (Figure 10). In any event, the larger percent inhibition of the late \( I_{Ca} \) may reflect a greater voltage-dependent inhibition of this component of the current. This suggests that quinidine may have a higher affinity for the open or inactivated state of calcium channels or that the channels must be opened to allow the drug access to a block site within the channel.

Basis for Effect of Quinidine on Action Potentials of Intact Myocardium and Isolated Myocytes

The action potentials of isolated myocytes differed characteristically from those of intact myocardium as previously reported. The \( V_{\text{mex}} \) values were higher in the isolated myocytes (317 versus 236 V/sec). Since the maximum diastolic potentials were not different, the larger \( V_{\text{mex}} \) of myocytes probably reflects the lack of electrotonic depression of this parameter resulting from the large current sink that exists in the intact myocardium. The isolated myocytes had a relatively negative maximum diastolic potentials were not different, the delay in the settling time of the dSEVC technique.

Quinidine produced parallel changes in the amplitude, overshoot, \( V_{\text{mex}} \), and plateau level of the action potential of both intact myocardium and isolated myocytes. Interestingly, quinidine prolonged the terminal phase of repolarization of the action potential in intact myocardium, but shortened the action potential of the isolated myocytes. The action potentials were either prolonged further in the intact myocardium or became prolonged beyond the control duration in the isolated myocytes after washout of quinidine, an effect also observed in canine Purkinje fibers.

The finding that quinidine inhibits inward \( Ca^{2+} \) and \( Na^+ \) currents as well as \( I_{K1} \) provides a basis for understanding the effects of quinidine on action potential configuration. For example, reduced inward currents may explain the negative shift in plateau voltage that occurs with exposure to quinidine. In addition, the abbreviation of the action potential in the plateau region causes a crossing-over of the action potential (Figure 2) in intact muscle and contributes to a shortening throughout the action potential in isolated myocytes. Although quinidine would be expected to prolong action potential duration because of its potent inhibitory effect on steady-state outward \( K^+ \) current (presumably \( I_{K1} \)), the actions of quinidine to inhibit these inward currents provide a counterbalance to the change in outward current that hastens repolarization in isolated myocytes.

The results in isolated myocytes are in contrast to the effects of quinidine in intact myocardium where quinidine prolongs terminal repolarization. Several explanations may be involved. First, it is possible that we are studying different cell types, one that occurs in the surface of the myocardium for intact preparations and another that is isolated from deep in the ventricular wall. Second, the presence of restricted intercellular spaces endows multicellular preparations with certain inhomogeneities due to accumulation and depletion of extracellular ions to which isolated myocytes are largely not as susceptible. It is possible, therefore, that the decrease in \( K^+ \) conductance that occurs with quinidine causes a decrease in cleft \( K^+ \) accumulation, which has further direct effects on \( K^+ \) conductances.

An important new finding of this study is that the effect of quinidine on \( I_{K1} \) appeared to reverse very slowly or not at all after washout of the drug. This action is distinctly different from the effects on other ionic currents (e.g., \( I_{Ca} \) and \( I_{Na} \)) for which the effects are fully reversible. This finding provides a basis for the prolongation of action potential duration during washout of quinidine in this and other studies.

Roden and Hoffman recently showed that quinidine produced a greater increase in \( A \overline{D}_{K1} \) at lower frequencies. The frequency-dependent effects of quinidine on inward currents combined with the frequency-independent effects on \( I_{K1} \) provide a possible explanation for their observation. At low frequencies, the tonic inhibition of outward current(s) would predominate producing greater action potential prolongation. At higher frequencies, there would be a larger use-dependent inhibition of the late inward currents, both...
Na⁺ and Ca²⁺, which would counterbalance and diminish the tonic action potential prolonging effect due to a frequency-independent inhibition of Iᵦ, and possibly Iᵦ. Quinidine might have additional frequency-dependent effects on action potential duration through inhibition of Iᵦ, depending on the kinetics of block of this current. These hypotheses require further study.

While this paper was in review, a similar study by Hiraoka et al. was published. In isolated guinea pig ventricular myocytes, quinidine prolonged action potential duration of myocytes stimulated at a frequency of 0.1 Hz. From voltage clamp experiments, they concluded that quinidine inhibited the delayed outward K⁺ current (Iᵦ, or Iₚ, reversibly), the inward rectifier (Iᵦ), irreversibly), and peak calcium current (Iₜ, reversibly). They also concluded that there was no effect of quinidine on the steady-state "window" Na⁺ current, but they did not look for slowly inactivating Na⁺ current, specifically.

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