Quinidine-Induced Action Potential Prolongation, Early Afterdepolarizations, and Triggered Activity in Canine Purkinje Fibers

Effects of Stimulation Rate, Potassium, and Magnesium

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Early afterdepolarization (EAD)-induced triggered activity is thought to contribute to the cardiac arrhythmogenic effects of several class I antiarrhythmic agents. The combination of quinidine therapy, bradycardia, and hypokalemia is known to predispose to torsade de pointes, which is a form of atypical polymorphous ventricular tachycardia commonly associated with long QT intervals. Recent clinical reports have shown suppression of quinidine-induced torsade de pointes with intravenous administration of magnesium sulfate. To further understand these relations, we used standard microelectrode techniques to examine the time course of quinidine-induced action potential prolongation, EAD, and triggered activity development and the dependence of these changes on \([K^+]_0, [Mg^{2+}]_0,\) and stimulation frequency in isolated Purkinje fiber preparations exposed to low concentrations of the drug. At slow stimulation rates, the quinidine-induced increase of action potential duration was slow to develop and failed to reach a steady state after 3 hours of exposure to the drug. EAD and EAD-induced triggered activity generally became apparent 70–90 minutes after adding the drug. Quinidine produced triggered activity in 10 of 22 preparations superfused with Tyrode’s solution containing normal \([K^+]_0 (3.5–4.0 \text{ mM})\) and in six other preparations when \([K^+]_0\) was reduced. In the presence of normal \([K^+]_0,\) two types of EAD and triggered activity were distinguished. In four of 10 preparations, this activity arose from phase 2 of the action potential; in eight of 10, it was associated with phase 3; and in two experiments, both types were present in the same preparation. The incidence of both forms of triggered responses depended greatly on the rate of stimulation. Triggered activity arising from phase 3 was always manifest at rates considerably slower than those giving rise to phase 2 activity. Both forms of triggered activity were sensitive to changes in the extracellular concentration of potassium and magnesium. Lower-than-normal levels of these electrolytes facilitated the manifestation of triggered activity, whereas elevated levels suppressed or caused a shift in the frequency-dependence of the activity. Phase 2, but not phase 3, EADs were abolished in response to increased \([Mg^{2+}]_0\). The data show a clear congruity between the conditions that predispose to torsade de pointes in the clinic and the conditions under which quinidine may induce triggered activity and marked action potential prolongation in isolated Purkinje fibers. The results add to our understanding of the mechanisms by which \([K^+]_0, [Mg^{2+}]_0,\) and heart rate may interact to influence the manifestation of quinidine-induced tachyarrhythmias. (Circulation 1989;79:674–686)

The contribution of early afterdepolarizations (EAD) and EAD-induced triggered activity to clinical arrhythmias is unclear. EAD activ-

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The contribution of early afterdepolarizations (EAD) and EAD-induced triggered activity to clinical arrhythmias is unclear. EAD activity has developed in isolated canine Purkinje fibers after a long period of exposure to quinidine.\(^1,2\) This activity becomes manifest at relatively slow stimulation rates and seems greatly increased when the \([K^+]_0\) is lowered.

In the clinic, the combination of bradycardia, hypokalemia, and quinidine is known to predispose to torsade de pointes, which is a form of atypical polymorphous ventricular tachycardia characterized in the electrocardiogram by “twisting” of the ventricular complexes around the isoelectric line. Torsade de pointes is commonly associated with QT
prolongation and has been reported to occur after the administration of several antiarrhythmic agents including quinidine, disopyramide, procainamide, and N-acetyl procainamide. Although the mechanisms underlying torsade de pointes are not well defined, EAD-induced triggered responses may be the initiating events. Recent in vivo studies have provided evidence for EAD and triggered activity as the underlying mechanism of polymorphic ventricular tachyarrhythmias in dogs exposed to cesium chloride or anthopleurin. Recent clinical and experimental studies also report the successful suppression of drug-induced torsade de pointes after intravenous magnesium sulfate administration.

In this study, we use standard microelectrode techniques to evaluate the effects of stimulation frequency and extracellular potassium and magnesium concentration on action potential duration, EAD, and triggered activity in canine Purkinje fibers exposed to therapeutic concentrations of quinidine for a period of 90 minutes or more.

**Methods**

Free running Purkinje fibers were isolated from the hearts of mongrel dogs of either sex after anesthesia with pentobarbital (30 mg/kg iv). Strands 2.5–6.0 mm long were placed in the tissue bath and superfused with Tyrode’s solution containing (mM): 137 NaCl, 4 KCl, 0.9 NaH2PO4, 20 NaHCO3, 1.8 CaCl2, and 0.5 MgSO4 (pH 7.4). The solution was bubbled with a 95% O2-5% CO2 mixture, and the temperature was maintained at 37±0.5°C. Transmembrane potentials were recorded from both ends of the Purkinje strand with glass microelectrodes filled with 2.7 M KCl (10–20 Dohm DC resistance) connected to a high-input impedance amplification system (World Precision Instruments, New Haven, Connecticut). The amplified signals were displayed on a Tektronix oscilloscope (Beaverton, Oregon) and photographed with a Grass kymographic camera (Quincy, Massachusetts) or recorded on an eight-channel tape recorder (Vetter, Rebersburg, Pennsylvania). The maximal rate of rise of the action potential upstroke was measured with a differentiator adjusted for linearity within the range of 80–800 V/sec. The fibers were stimulated at one end with rectangular pulses (1–3 msec duration and two to three times threshold intensity) applied through a pair of thin silver electrodes insulated except at the tips. A 45-minute equilibration period was permitted before control measurements were obtained.

The preparations were stimulated over a wide range of basic cycle lengths (300–8,000 msec) starting at the longest basic cycle length permitted by the intrinsic automaticity of the preparation. Each frequency was maintained until the action potential duration (APD) achieved a steady state before collecting data (maximum of 2 minutes). After each scan of stimulation frequencies, the rate was returned to the longest basic cycle length used in the protocol until the next scan was performed. This approach minimized the time required to achieve a steady state of action potential variables, a problem commonly encountered during deceleration of the stimulation rate in fibers exposed to quinidine.

In most experiments, the frequency scan was repeated two or three times for assessment of reproducibility before testing the effect of changes in potassium or magnesium concentration. Curves relating the incidence of quinidine-induced triggered activity as a function of the basic cycle length were constructed for activity recorded before and after the changes in [K+]0 and [Mg2+]0 and after the electrolytes were restored to normal levels. In all graphs, curves were fitted by eye.

The effects of changes in [K+]0 and [Mg2+]0 were assessed in different preparations. A period of 15–20 minutes was allowed after each change of [K+]0 or [Mg2+]0 before collecting data. In experiments designed to quantify the effects of [Mg2+]0 on action potential variables, we selected the longest stimulation cycle length (between 1,500 and 3,000 msec) that yielded a consistently stable action potential morphology (after quinidine administration). That frequency was then maintained for the duration of the experimental protocol.

In most experiments, impalements were maintained in the same cell for the duration of the study. When reimpalement was necessary, data were included for analysis only when the action potential characteristics of the reimpaled cell were nearly identical to those of the cell before the loss of impalement as evidenced by superimposing old and new action potential traces on a storage oscilloscope with a recheck at time of analysis. Measurements of the maximal rate of rise (Vmax) of the action potential are reported only for experiments in which an impalement of the same cell was maintained for the duration of the experimental protocol.

We define EADs and triggered activity according to Cranefield and Damiano and Rosen. An EAD was considered an afterpotential that interrupts or delays the normal repolarization of the action potential. As noted by Cranefield, some EADs do not show an actual depolarization but only a delay of repolarization. A triggered action potential was defined as a second nonstimulated upstroke arising from the EAD and exhibiting an amplitude greater than 10 mV. Triggered beats were classified as phase 2 or phase 3 events based on the phase of the action potential from which they arose and in doubtful cases based on their takeoff potential. Triggered activity with takeoff potentials more positive than –30 mV were classified as phase 2 events, whereas those with a takeoff more negative than –30 mV were classified as phase 3 events. EADs were similarly classified based on the potential of the inflection point at which the repolarization process was interrupted.

Quinidine HCl was prepared fresh weekly and kept refrigerated as a stock solution of 1 mg/ml. The drug was added to the Tyrode perfusate to obtain
final concentrations of 0.5–1 μg/ml. The K⁺ and Mg²⁺ levels were modified over a range of 2.7–5.0 mM and 0.5–5 mM, respectively.

The APD was measured at 50% or 90% repolarization in all cases. Statistical analysis was performed with Dunnett’s test or analysis of variance followed by Duncan’s multiple range test as indicated. Differences with p values less than 0.05 were considered significant.

Results

The relation between APD and cycle length is well known. The effect of quinidine to alter the slope of this relation and to induce EAD and triggered activity was recently reported in an elegant study by Roden and Hoffman.² The time course of quinidine’s action to prolong APD, particularly under the conditions that promote EAD activity, however, is not well defined. The experimental protocols used by Roden and Hoffman were based on the assumption that a steady-state effect of quinidine may be achieved within 30 minutes. In preliminary experiments designed to assess the time course of APD prolongation, we were unable to define a steady state for quinidine’s effects at slow rates of stimulation; APD continued to prolong 3 hours after the introduction of quinidine (Figure 1). EAD and triggered activity attended by marked prolongation of APD generally became manifest 70–90 minutes after adding quinidine. The data reported in the present study, therefore, were collected 90 or more minutes after introducing the drug.

The range of quinidine concentrations used (0.5–1.0 μg/ml) was chosen to reflect therapeutic plasma levels of free drug after correction for protein and lipoprotein binding (0.4–1.5 μg/ml).¹⁵–¹⁹

Action Potential Duration in the Absence of Triggered Activity

In six of 22 isolated Purkinje preparations superfused with normal Tyrode’s solution containing 3.5–4.0 mM KCl, quinidine failed to induce triggered activity. In these fibers, we were able to define the relation between APD and stimulation rate throughout a wide range of frequencies. Figure 2 illustrates a representative example of activity recorded before and 90 minutes after adding quinidine (0.5 μg/ml). Shown are transmembrane recordings obtained with stimulation at basic cycle lengths of 300 (left) and 2,000 msec (right). In this example, quinidine produced a 7.2% prolongation of APD at a basic cycle length of 300 msec (176–189 msec), but a 51.5% increase of APD at a basic cycle length of 2,000 msec (305–462 msec). In this and five similar experiments, the effect of quinidine on APD was much greater at the longer basic cycle lengths.

In some cases, as in the example presented in Figure 3, the development of a pronounced EAD during phase 3 produced a dramatic prolongation of APD. Upper and lower traces represent transmembrane potentials recorded from opposite ends of the Purkinje fiber. At a basic cycle length of 8,000 msec, alternating short and long diastolic intervals resulted in alternating short and long action potentials.
Afterdepolarizations

Early Dependence of Frequency of the quinidine Tyrode's, achieving upon failed EAD and were observed triggered activity. Occasionally, as illustrated in Panel B, the response failed to repolarize. The fibers were generally inexcitable during the prolonged repolarization phase or upon achieving a second stable resting state.

Early Afterdepolarizations and Triggered Activity

In 10 of 22 preparations superfused with normal Tyrode's, quinidine (0.5–1.0 μg/ml) produced triggered activity. In six other preparations, a reduction of [K⁺]₀ (2.5–3.2 mM) was required to induce triggered activity. In the latter group, triggered activity appeared within 10 minutes after the decrease in [K⁺]₀ and was abolished when normal [K⁺]₀ was restored.

At normal K⁺ levels (3.5–4.0 mM), two types of EAD and triggered activity could be distinguished. In four of the 10 preparations, oscillatory activity accompanied by one or more spikes appeared at the level of the action potential plateau (Figure 4A). In eight of the 10 preparations, EADs were observed at more negative potentials, occurring during phase 3 of the action potential and giving rise to single or multiple triggered beats (Figure 4B). In two experiments, both forms were manifest in the same preparation (Figure 4C). EAD and triggered activity at the plateau levels (phase 2 of the action potential) were never observed in preparations exposed to low [K⁺]₀.

Frequency Dependence of Triggered Activity

Triggered activity arising from phase 2 and phase 3 of the action potential displayed different sensitivities to changes in stimulation rate. The frequency dependence of activity arising at the level of the plateau is illustrated in Figure 5. The two traces in each panel represent transmembrane activity recorded from opposite ends of a Purkinje fiber. At a basic cycle length of 800 msec, only minor oscillations were observed at the plateau level. At basic cycle lengths of 1,000 and 1,200 msec, discernible spikes appeared; triggered activity represents 33% and 50%
The results of the two experiments illustrated in Figures 5 and 6 are presented graphically in Figure 7 with the results of seven additional experiments. The incidence of triggered activity (percentage of total activity) is plotted as a function of the basic cycle length. Triggered activity arising from phase 3 of the action potential always appeared at rates considerably slower than those giving rise to phase 2 activity. In two preparations exhibiting both types of triggered activity (indicated by bars), the basic cycle length at which phase 2 triggered activity appeared was several hundred milliseconds shorter than the basic cycle length at which phase 3 activity became manifest. One preparation (not shown) displayed phase 3 triggered activity at basic cycle lengths of 5,000–10,000 msec. Overall, phase 2 triggered activity first appeared at a basic cycle length of 990±216 msec (mean±SD), whereas phase 3 triggered activity became manifest at an average basic cycle length of 2,371±675 msec (3.5–4.0 mM [K+]0).

Effects of [K+]0 on Triggered Activity

In six of 22 experiments, triggered activity appeared only when the potassium chloride concentration of the Tyrode’s solution was reduced below 3.5 mM. In two experiments in which triggered activity appeared during exposure to normal Tyrode’s solution (4 mM KCl), reduction of [K+]0 greatly increased triggered activity manifestation at the higher frequencies through a shift in the rate dependence of triggered activity incidence (Figure 8). The incidence of triggered activity is plotted as a function of the basic cycle length. After 100 minutes of superfusion with normal Tyrode’s solution (4 mM [K+]0) containing quinidine (0.5 μg/ml), trig-
triggered activity (phase 3) appeared only at low stimulation frequencies (basic cycle length = 3,500–4,500 msec). Seven minutes after the reduction of $[K^+]_0$ to 3.5 mM, triggered activity was observed at shorter basic cycle lengths (2,500–4,000 msec). Further reduction of $[K^+]_0$ to 3.0 mM resulted in a further shift of the curve to the left.

**Effects of $[Mg^{2+}]_0$ on Triggered Activity**

The magnesium sulfate concentration of the Tyrode’s solution was varied from normal (0.5 mM) to 5 mM in eight experiments. In the example illustrated in Figure 9, a slight increase of $[Mg^{2+}]_0$ from 0.5 to 1.0 mM 1) totally suppressed triggered activity at the faster stimulation rates (basic cycle length≤1,500 msec), 2) decreased the incidence of triggered responses at slower rates, and 3) abolished runs of triggered activity (spontaneous beating). The increase of magnesium sulfate concentration to 1.0 mM produced qualitatively similar effects in all preparations exposed to normal $[K^+]_0$ ($n = 5$). These effects of magnesium sulphate were readily reversed. In preparations in which lower concentrations of $K^+$ (2.7–3.2 mM) were used to elicit triggered activity, higher concentrations of $MgSO_4$ (1.5–3.0 mM) were needed to modify the manifestation of this activity. High levels of $Mg^{2+}$ (≥3 mM) completely suppressed triggered activity in all preparations.

**Effects of $[Mg^{2+}]_0$ on Action Potential Variables and Early Afterdepolarizations**

In the absence of quinidine, elevation of $[Mg^{2+}]_0$ from the normal concentration of 0.5 mM to a concentration of 3 mM produced no significant changes in action potential variables (Figure 10 and Table 1). Higher $[Mg^{2+}]_0$ (5 mM) caused a significant depolarization of the maximum diastolic potential. Other action potential variables (amplitude, $APD_{90}$, $APD_{90}$, and $V_{max}$) diminished with progressive increases of $[Mg^{2+}]_0$, but these changes were not significant (Table 1).

After exposure to quinidine, progressive increases of $[Mg^{2+}]_0$ from 0.5 to 3.0 mM again produced small decreases in the action potential variables; all but the maximum diastolic potential failed to achieve statistical significance. At still higher $[Mg^{2+}]_0$ (5 mM), significant decreases in the maximum diastolic potential, amplitude, and APD at 90% were observed (Table 1).

Changes of $[Mg^{2+}]_0$ produced different effects on the two types of EAD. In the case of phase 3 events, when the EAD was barely perceptible, increasing $[Mg^{2+}]_0$ led to its disappearance (Figure 10). However, when a prominent EAD was mani-
fest, increases of Mg\(^{2+}\) even to a concentration of 5 mM did not attenuate the EAD to any great degree (Figure 11). The action potential abbreviation produced by increasing [Mg\(^{2+}\)]\(_0\) to 5 mM seems largely due to a depression of the late portion of phase 2. In contrast, high concentrations of Mg\(^{2+}\) (3–5 mM) completely abolished phase 2 EADs in all cases (three preparations).

Great care was taken in these studies to exclude any experiments in which the pattern of the action potential was not stable before the changes of [Mg\(^{2+}\)]\(_0\) or in which a substantial difference existed between the action potential variables recorded at the start of the experimental protocol at a [Mg\(^{2+}\)]\(_0\) of 0.5 mM and at the end of the protocol upon return to a [Mg\(^{2+}\)]\(_0\) of 0.5 mM.

**Discussion**

In the present study, we characterize the manifestation of quinidine-induced triggered activity and APD prolongation concerning dependence on the rate of stimulation and on dependence of the potassium and magnesium concentration of the superfusate. In contrast to previous reports,\(^2,20\) we found that quinidine-induced APD prolongation and triggered activity in most preparations were slow to develop and did not reach a steady state after 3 hours of exposure to the drug. Quinidine-induced increases of APD showed a steep rate dependence with a much greater prolongation occurring at the longer basic cycle lengths. This behavior is consistent with that observed with a variety of agents that act to alter APD as reported by Nattel and Zeng\(^{21}\)

![Figure 9](image-url)  
**Figure 9.** Tracings of effects of [Mg\(^{2+}\)]\(_0\) on triggered activity arising from phase 3 of the action potential. Each panel shows transmembrane activity recorded from opposite ends of a Purkinje fiber. Panel A: Recorded 90 minutes after superfusion with normal Tyrode's solution containing quinidine (0.5 \(\mu\)g/ml). Patterns of trigeminy, bigeminy, and multiple triggered responses were observed at BCLs of 1,500 and 2,000 msec and during spontaneous beating, respectively. Panel B: Recorded 7 minutes after increasing MgSO\(_4\) concentration to 1.0 mM. Triggered responses were suppressed at a BCL of 1,500 msec and reduced (from bigeminy to trigeminy) at a BCL of 2,000 msec. Repetitive triggered responses were no longer observed.

![Figure 10](image-url)  
**Figure 10.** Tracings of effects of [Mg\(^{2+}\)]\(_0\) on action potential variables. Transmembrane activity recorded from an isolated Purkinje preparation superfused with Tyrode's containing 0.5, 1.0, and 5.0 mM MgSO\(_4\) before (solid lines) and after (dashed lines) 180 minutes of exposure to quinidine (0.5 \(\mu\)g/ml) (3.5 mM [K\(^{+}\)]\(_0\)). BCL, 2,000 msec.
and Varro and coworkers. The large interfiber variability of APD values in control was accentuated after exposure to quinidine, especially at the slower stimulation rates (Figure 1). The drug-induced increase in variance was due to differences in the responsiveness of individual fibers to the drug. Fibers exhibiting long APDs and a steep APD-rate relation in control showed the largest increases of APD in response to quinidine (unpublished observation from this laboratory).

EAD and triggered activity were observed only after long periods of exposure to the drug and at slow stimulation rates, conditions under which the effect of quinidine on APD is most pronounced (Figures 1 and 2). Nevertheless, we were unable to clearly define a relation between the degree of quinidine-induced APD change and triggered activity induction. Nearly half of the preparations developed triggered activity when bathed in Tyrode’s solution containing normal [K+]o. The reason for the inhomogeneous response to the drug is not clear but may be due, in part, to anatomic factors (e.g., origin, size, and degree of muscle infiltration). At low [K+]o, 73% of the preparations developed EAD-induced triggered activity after long exposure to low (therapeutic) concentrations of quinidine. The inability to induce triggered activity in some of the remaining fibers was due to the accentuation of automaticity at the lower [K+]o, which precluded the achievement of a basic cycle length sufficiently long to allow for the expression of triggered activity.

In most experiments, we obtained simultaneous intracellular recordings from opposite ends of the fibers (Figures 3–6 and 9) to rule out the possibility that the observed EAD or triggered activity may be due to electrotonic interactions or reentrant activity within the tissue. In some experiments, especially when long fibers were used, we mapped the entire fiber to exclude this possibility. Disparate activity was recorded along the fiber length in only two preparations; these were not included in the data evaluation.

Two different types of EAD and triggered activity were observed. Triggered activity arising from phase 2 of the action potential (Figure 4A) occurred at relatively short basic cycle lengths (Figures 5 and 7), some as short as 800 msec. In all cases, a slowing of the stimulation rate led to an increase in

**TABLE 1. Effects of [Mg²⁺]₀ on Action Potential Variables in the Absence and Presence of Quinidine**

<table>
<thead>
<tr>
<th>[Mg²⁺]₀</th>
<th>Maximum diastolic potential</th>
<th>Amplitude</th>
<th>APD₉₀</th>
<th>APD₉₀</th>
<th>Vₘₕₙₐₓ</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.5</td>
<td>−89.0 ± 1.0</td>
<td>120.2 ± 3.4</td>
<td>276.0 ± 45.5</td>
<td>361.0 ± 44.4</td>
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<td>1.0</td>
<td>120.6 ± 1.3</td>
<td>196.2 ± 3.2</td>
<td>270.0 ± 40.5</td>
<td>353.0 ± 45.8</td>
<td>554.0 ± 118.2</td>
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<td>2.0</td>
<td>187.4 ± 1.5</td>
<td>258.0 ± 39.5</td>
<td>340.0 ± 33.7</td>
<td>544.0 ± 111.6</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>117.4 ± 4.2</td>
<td>251.0 ± 30.7</td>
<td>339.0 ± 34.6</td>
<td>534.0 ± 100.6</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>−84.8 ± 2.5*</td>
<td>116.0 ± 4.9</td>
<td>243.0 ± 27.5</td>
<td>326.0 ± 40.2</td>
<td>518.0 ± 107.6</td>
</tr>
</tbody>
</table>

Quinidine

<table>
<thead>
<tr>
<th>[Mg²⁺]₀</th>
<th>Maximum diastolic potential</th>
<th>Amplitude</th>
<th>APD₉₀</th>
<th>APD₉₀</th>
<th>Vₘₕₙₐₓ</th>
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<tr>
<td>0.5</td>
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<td>119.2 ± 2.6</td>
<td>359.0 ± 67.9</td>
<td>635.0 ± 91.8</td>
<td>548.0 ± 110.4</td>
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<tr>
<td>1.0</td>
<td>−87.0 ± 1.2</td>
<td>118.0 ± 2.1</td>
<td>350.0 ± 61.2</td>
<td>628.0 ± 92.1</td>
<td>546.0 ± 117.9</td>
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<tr>
<td>2.0</td>
<td>−85.2 ± 2.2</td>
<td>117.4 ± 1.8</td>
<td>341.0 ± 57.3</td>
<td>600.0 ± 77.1</td>
<td>532.0 ± 109.0</td>
</tr>
<tr>
<td>3.0</td>
<td>−84.4 ± 2.6†</td>
<td>116.0 ± 2.7</td>
<td>325.0 ± 61.9</td>
<td>599.0 ± 16.7</td>
<td>532.0 ± 109.0</td>
</tr>
<tr>
<td>5.0</td>
<td>−82.8 ± 3.7*</td>
<td>113.8 ± 4.8†</td>
<td>284.0 ± 44.4</td>
<td>530.0 ± 82.2†</td>
<td>512.0 ± 99.0</td>
</tr>
</tbody>
</table>

Data are mean ± SD in msec obtained from five Purkinje fiber preparations.

After control measurements, the fibers were exposed to quinidine (0.5 μg/ml) for 180–360 minutes. Each preparation was stimulated at the longest basic cycle length at which the action potential pattern remained stable before varying the [Mg²⁺]₀. Basic cycle length is 1,500–3,000 msec Data were collected approximately 15 minutes after each change of [Mg²⁺]₀.

APD₉₀ and APD₉₀, action potential duration at 50% and 90% repolarization; Vₘₕₙₐₓ, maximal rate of rise of the action potential upstroke.

*p < 0.01, †p < 0.05 compared with value at 0.5 mM [Mg²⁺]₀; Dunnett’s test.

**FIGURE 11. Tracings of effect of [Mg²⁺]₀ on a quinidine-induced early afterdepolarization. Superimposed action potentials recorded from a Purkinje fiber superfused with Tyrode’s solution containing 0.5, 1.0, and 5.0 mM MgSO₄ after exposure to quinidine (0.5 μg/ml) for 240 minutes (2.7 mM [K+]₀). BCL, 2,000 msec. The phase 3 EAD is largely unaffected by the changes in Mg²⁺ levels. The predominant effect of high [Mg²⁺]₀ is to depress phase 2 and slightly abbreviate the action potential.**
the incidence of this activity (Figures 4A, 5, and 7). Triggered activity arising from phase 3 was similarly sensitive to changes of stimulation rate but throughout a lower range of frequencies. Both types of EAD have been observed in canine Purkinje fibers exposed to cesium10,14 and to hypoxia plus epinephrine.23 Quinidine-induced triggered activity at the level of the action potential plateau was also observed by Roden and Hoffman in canine Purkinje fibers exposed to 10 μM quinidine and 2.7 mM [K+]o (basic cycle length >4,000 msec); however, the takeoff potentials reported (∼44 to ∼55 mV) were considerably more negative than those of the responses defined as phase 2 triggered activity in the present study (positive to ∼30 mV).

Both types of triggered activity were sensitive to changes in extracellular concentrations of potassium and magnesium. Reduced levels of these electrolytes facilitated the manifestation of triggered activity, whereas elevated levels suppressed the activity. In some preparations, the reduction of [K+]o increased normal automaticity to the extent that triggered activity could not be unmasked.

Slight elevations of extracellular magnesium sulfate concentrations induced a reversible shift in the frequency dependence of triggered activity manifestation to lower frequencies with no significant effects on action potential pattern. Higher concentrations of magnesium sulfate completely suppressed triggered activity but also depressed action potential variables. The effectiveness of Mg2+ to suppress cesium-induced triggered activity was recently documented in vivo and in vitro studies by Bailie and coworkers.10

Administration of magnesium sulfate in the clinic may lead to plasma magnesium concentrations as high as 5.2 mg per 100 ml; higher levels (12 mg/100 ml) are reported in patients with nephritis. No adverse effects have been reported in animals with levels up to 6 mg/dl. Our concentrations of 0.5, 1.0, and 5.0 mM are equivalent to 1.2, 2.4, and 12 mg/dl, respectively.

Ionic Mechanisms

Voltage clamp studies in multicellular and single cell cardiac preparations report quinidine inhibition of the fast sodium inward current (INa), delayed rectifier current (IKr, IKs: outward K+ current), pacemaker current (IF, ICa), slow inward current (Ica), and steady-state or “window” sodium current.24-27 The effect of quinidine to prolong APD and produce EAD-induced triggered activity may be ascribed chiefly to the drug’s action to inhibit the delayed rectifier because diminution of the other currents would be expected to abbreviate APD and prevent the emergence of EAD and triggered activity or exert little effect. Imaizumi and Giles recently reported quinidine’s effect to inhibit the transient outward current in isolated rabbit myocytes. The possible contribution of this drug effect to the manifestation of EADs and triggered activity remains to be elucidated.

Interventions that reduce sodium current (tetrodotoxin) and low [Na+]o diminish or abolish quinidine-induced EADs and triggered activity,2 whereas those that block the calcium INa have been shown to suppress quinidine-induced triggered activity but to exert only minor effects on EADs (referred to as the “secondary plateau”).29

Quinidine, at a concentration of 0.5 μg/ml and at slow stimulation rates, exerts no effect on Vmax of the action potential, yet the effect on phase 3 of the action potential is remarkable (unpublished observations from this laboratory; see also Roden and Hoffman2). This observation indicates that marked inhibition of IK by quinidine may be effected with little if any inhibition of INa. The independence of these two actions of the drug is further supported by the difference in the time course of these effects. With higher quinidine concentrations or faster frequencies, both, the drug clearly depresses Vmax; the onset of this effect is relatively rapid with a steady-state effect achieved within 60 minutes.30 As for quinidine’s actions on phase 3, we failed to observe a steady-state effect after 3 hours. Moreover, EAD and triggered activity were generally evident only after 70–90 minutes of exposure to quinidine. In preliminary washout experiments, we observed rapid washout of quinidine’s effect on Vmax with a very slow washout of the drug’s effects on phase 3. In some cases, a 90-minute lag occurred between the start of washout and the initial APD shortening and EAD diminution. These results are consistent with those reported by Nattel and Bailey,30 who concluded that the quinidine-induced change in the slope of phase 3 was due to a mechanism different from that producing phase 0 and phase 2 changes.

Our observation of a slow progressive development of quinidine-induced changes in action potential variables (at low frequencies) has not been previously reported. In a corollary study, we recently showed a similar slow progressive development of quinidine-induced action potential prolongation and EADs in canine ventricular muscle.31 To obtain a better understanding of the factors responsible for the slow development of these electrophysiologic actions of quinidine, we quantified tissue uptake of the drug in a parallel study.32 The time course of quinidine uptake into muscle was well fitted by a biexponential relation with widely disparate time constants. The fast component, thought to represent drug equilibration within the extracellular space, displayed time constants of several minutes, whereas the slower process, believed to characterize the intracellular accumulation of the drug, displayed time constants of several hours. The biexponential time course of tissue uptake of quinidine paralleled the time course of changes in APD, suggesting that the slow developments of quinidine’s effect on action potential repolarization may, in large part, be attributed to the slow intracellular accumulation of the drug. After 7.5 hours of exposure to quinidine (1 μg/ml), concentrations of the drug in tissue were more than ninefold
higher than those in the perfusate. Tissue uptake of quinidine into canine Purkinje fibers is difficult to quantify because of the limited amount of tissue available for assay. In preliminary experiments with calf Purkinje fibers, we found a qualitatively similar time course of drug uptake.

**Increase of Quinidine’s Actions on Phase 3 at Slow Stimulation Rates**

Quinidine block of the sodium channels is known to be use or tachycardia dependent. After an abrupt acceleration of the stimulation rate, \( V_{\text{max}} \) decreases with each successive beat until a steady state is achieved. The steady-state level is a function of the stimulation rate. This phenomenon has been explained with various models in which the affinity or accessibility of the drug to the sodium channel is modulated by membrane potential or the state of the channel. Quinidine’s action to depress \( V_{\text{max}} \) in a use- and rate-dependent manner is generally thought to be due to the drug’s ability to block activated sodium channels during phase 0 of the action potential. Recovery from block is most rapid at more negative potentials, achieved after repolarization, when most of the sodium channels are in the resting state.

In contrast, quinidine’s effect on APD is clearly bradycardia and disuse dependent. APD prolongation and EAD development are greatly facilitated when the stimulation rate is slowed. After an abrupt deceleration of basic drive, APD prolongation and EAD manifestation increase progressively until a new steady state is achieved. The normal mechanisms underlying the rate dependence of APD clearly must contribute to this process; however, one is tempted to speculate that quinidine’s interaction with the delayed rectifier \( I_K \) is opposite to its interaction with the sodium channel (inward current) in the sense that the drug may preferentially block the rested channels (during diastole) and unblock (dissociate) during the action potential when the channels are in the active state. In support of this hypothesis, a report by Roden and coworkers indicates that quinidine block of the \( K^+ \) channels is indeed voltage dependent and that preferential block occurs at more negative potentials.

Progressive inhibition of \( I_K \) with deceleration would be expected to unmask other currents active during repolarization. Prominent among these are the steady-state or “window” sodium current and the slowly inactivating sodium current described by Gintant and coworkers and Saikawa and Carmeliet. Not only would the relative contribution of these currents to the total membrane current during phase 3 be increased as outward current declines with deceleration, but the absolute level of these inward currents may also be augmented due to a lesser quinidine block of the sodium channels at the slower rates. The voltage dependence of the steady-state sodium current is such that it peaks at potentials at which phase 3 EADS appear.

Another factor contributing to the increased manifestation of EAD and triggered activity at slower rates may be the level of \( Na^+ -K^+ \) pump current. A decreased \( Na^+ \) influx attending deceleration would be expected to decrease the activity of the \( Na^+ -K^+ \) pump and thereby diminish the intensity of the outward current generated by the electrogenic pump.

Quinidine-induced APD prolongation and EAD generation, thus, may be viewed as secondary to a rebalancing of the outward and inward currents normally active during repolarization of the action potential. Triggered responses arise when the net current becomes inward and their firing is facilitated by a regenerative increase in inward sodium and calcium currents. This hypothesis is consistent with the results of computer simulations reported by Coulombe et al.

**Effect of \([K^+]_0\) and \([Mg^{2+}]_0\) on Quinidine-Induced Changes in Phase 3**

Changes in \([K^+]_0\) modify several ionic currents. A reduction of \([K^+]_0\) is known to diminish \( I_T \), \( I_K \) (\( Na^+ -K^+ \) pump current) and \( I_{K1} \) (steady-state outward current). The net effect of these changes is to further decrease the relative contribution of outward current during the repolarization process, thus further slowing repolarization and facilitating EAD and triggered activity. Changes of \([K^+]_0\) have been shown to cause a parallel shift in the APD-frequency relation in the presence and absence of quinidine. This finding suggests that the predominant effect of changing \([K^+]_0\) is to alter the time-independent inward rectifier (\( I_{K1} \)).

Under control conditions, small elevations of \([Mg^{2+}]_0\) produced no significant changes in action potential variables. These findings agree with previous in vitro studies with canine Purkinje fibers. In humans, magnesium in doses sufficient to increase serum levels to 5.4 mg/dl does not alter the QT interval or the atrial or ventricular refractory periods during sinus rhythm. The depolarization observed with higher concentrations of magnesium has also been observed by others.

The effect of \( Mg^{2+} \) to diminish or suppress triggered activity may be related to a “stabilizing” or surface charge effect. Elevated \( Mg^{2+} \) levels have been shown to shift the threshold potential to less negative values in frog sartorius muscle. Magnesium ions produce a membrane stabilizing effect similar to but less effective than that of \( Ca^{2+} \) in the squid axon (see also Weidmann). Assuming a similar effect of \( Mg^{2+} \) in canine Purkinje fibers, it may be reasonable to suggest that triggered activity inhibition by \( Mg^{2+} \) may be secondary to an increase of the threshold current requirements for activation of the triggered response (i.e., a shift of the threshold potential to more positive values). In addition, \( Mg^{2+} \) may act to suppress triggered activity through an inhibition of \( I_{Na} \) if its effect on \( I_{Na} \) in canine Purkinje fibers is similar to that reported for guinea pig papillary muscle and frog atrial fibers.
bition of I_\infty could also account for the depression of phase 2 observed with high levels of Mg^{2+} (Figures 10 and 11). The effects of I_\infty blockers are similar to those produced by Mg^{2+} in that both suppress triggered activity but not EADs that arise from phase 3 of the action potential.29,50

**Triggered Activity and Early Afterdepolarizations Arising From Phase 2 of the Action Potential**

The basis for quinidine-induced triggered activity and EADs arising at the level of the action potential plateau is far from clear. Marban and coworkers\(^5\) showed that cesium-induced phase 2 EADs can be abolished by I_\infty blockers but not by agents that diminish the intracellular calcium activity of ferret ventricular muscle. Moreover, calcium channel agonists and elevated [Ca^{2+}], induced triggered activity (referred to as “potentiated EADs”) in the cesium-treated preparations. The investigators concluded that under these conditions EADs arise as a direct consequence of calcium entry through the sarclemmal calcium channels and that unlike delayed afterdepolarizations they do not require elevated [Ca^{2+}] to manifest. In canine Purkinje fibers pretreated with quinidine, catecholamines accentuate phase 2 but not phase 3 EADs and increase the manifestation of both phase 2 and phase 3 triggered activity (unpublished observation from this laboratory). Like calcium channel blockers, magnesium greatly diminishes the amplitude of phase 2, but not phase 3, EADs but suppresses both types of triggered activity in quinidine-treated fibers. These differences in the responsiveness of phase 2 and phase 3 EADs to magnesium and to calcium channel agonists and antagonists may be a consequence of the membrane voltage at which these responses appear. Phase 2 EADs occur at potentials coincident with the I_\infty activation range, whereas phase 3 EADs generally occur below this range. Alternatively, different mechanisms may be invoked to explain the two types of activity. The idea of a unified mechanism is supported by the observation that a wide variety of EAD-inducing agents produce both types of EAD whether or not they act through inhibition of the delayed rectifier (quinidine), the inward rectifier (cesium), by slowing the inactivation of the sodium channels (anthopleurin),11 or by other mechanisms (amiloride).52 In all cases, the balance of currents is shifted in an inward direction during the repolarization phases of the action potential. A better mechanistic understanding of these phenomena awaits further study.

**Clinical Correlation**

Quinidine-induced ventricular tachyarrhythmias, in particular the polymorphous ventricular tachycardia known as torsade de pointes, are frequently associated with hypokalemia, therapeutic plasma levels of quinidine, and QT interval prolongation.3-6 In addition, the initiation of torsade de pointes is usually preceded by a slow heart rate or a long pause. Quinidine-induced torsade de pointes and other ventricular tachycardias associated with long QT intervals1,2,7,8,20 may be precipitated by triggered responses arising from EADs that develop in Purkinje fibers. Recent clinical studies1,2,5,3 also report the successful treatment of quinidine-induced torsade de pointes with intravenous administration of magnesium.

Our data show a clear congruity between the conditions that predispose to torsade de pointes and the conditions under which quinidine induces triggered activity and marked prolongation of the action potential in isolated Purkinje fibers. Moreover, alterations that protect against torsade de pointes in the clinic (Mg^{2+}, K^{+}, and pacing) prevent or diminish quinidine-induced triggered activity and APD prolongation in vitro.

Extrapolation of these observations to the understanding of the arrhythmogenic actions of quinidine in vivo, however, must be approached with some care. The available data suggest that in the presence of quinidine, slow activation rates especially when combined with low [K^{+}]\(_o\) levels can result in 1) extrasystolic or parasystolic activity due to EAD-induced triggered activity, 2) a marked dispersion of refractoriness between the conduction system and the myocardium due to a much greater lengthening of the APD of Purkinje fibers compared with that of ventricular muscle,52 and 3) conduction block due to the development of a second resting state at more depolarized levels (Figure 3). Thus, bradycardia or hypokalemia or both attending the use of quinidine may facilitate the development of triggered activity and set the stage for a variety of reentrant arrhythmias.

The beneficial effects of Mg^{2+} in the suppression of digitalis-induced arrhythmias and dysrhythmias attending myocardial ischemia, alcoholism, and diuretic therapy have long been known.55-58 Only recently has magnesium sulfate been shown to be effective in the treatment of drug-induced torsade de pointes.10,12,53 Our results indicate that Mg^{2+} may act by suppressing the EAD-induced triggered activity that may be responsible for initiating episodes of torsade de pointes.

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