Action Potential Alternans and Irregular Dynamics in Quinidine-Intoxicated Ventricular Muscle Cells
Implications for Ventricular Proarrhythmia

Hrayr S. Karagueuzian, PhD; Steven S. Khan, MD; Kichol Hong, MD; Yoshinori Kobayashi, MD; Timothy Denton, MD; William J. Mandel, MD; and George A. Diamond, MD

Background. Cardiac cells display rate-dependent beat-to-beat variations in action-potential duration (APD), action potential amplitude (APA), and excitability during periodic stimulation. We hypothesized that quinidine causes a marked increase in the variability of APD, APA, and excitability of ventricular cells isolated from quinidine-toxic, arrhythmic ventricles.

Methods and Results. Action potentials were recorded from right ventricular endocardial tissues (2×1 cm, <2 mm thick) isolated from dogs in which ventricular tachycardia and ventricular fibrillation (VT/VF) were induced with intravenous quinidine (80–100 mg/kg) over a 5-hour period in vivo (n = 7). As the basic cycle length (BCL) of stimulation was progressively shortened, rate-dependent variations in APD and APA occurred. The initial dynamic change was alternans of APD and APA that could be either in or out of phase between two cells. The magnitude of alternans was a function of the BCL and the strength of the stimulation current. At critically short BCLs, irregular APD and APA behavior emerged in the quinidine-intoxicated cells. In control cells (n = 16) isolated from three nontreated dogs, APD and APA remained constant at all BCLs tested (2,000–300 msec). Quinidine increased the slope of the APD restitution curve compared with control. The observed quinidine APD restitution curve was fitted with a biexponential equation, and computer simulation using the fitted restitution curve reproduced the aperiodic APD seen in the quinidine toxic cells during periodic stimulation. Thus, the observed irregular APD behavior was predictable from the restitution curve.

Conclusions. Quinidine toxicity increases the temporal and spatial variability of APD and APA in the ventricle that may promote the initiation of reentrant VT/VF in vivo. The slope of the APD restitution curve provides a method to quantitate inhomogeneities in repolarization time and could be a useful marker for proarrhythmia. (Circulation 1993;87:1661–1672)

Key Words • action potential duration restitution • bifurcation • proarrhythmia • chaos quinidine

Sir Thomas Lewis observed in 1911 that “heart alternation occurs under two circumstances. It is seen when the cardiac muscle is not of necessity altered structurally, as an accompaniment of great acceleration of the rate of rhythm. It is also found when the pulse is of normal rate, and under such circum-
stances the muscle is either markedly degenerate or the heart shows evidence of embarrassment as a result of poisoning or some other factor.”

Although Lewis’ experimental observations were made more than 80 years ago, the importance of beat-to-beat changes in cardiac cellular electrical activity became appreciated only recently. It was found that quinidine could exert qualitatively variable effects on cardiac excitability during a modest elevation of extracellular potassium ion concentration ([K]eo). In fact, it was shown that quinidine could either increase or decrease the excitability of isolated sheep cardiac Purkinje fibers. This biphasic response is common in nonlinear dynamical systems and may conceivably lead to or be associated with other qualitative electrophysiological changes such as alternations in action potential duration (APD) and/or action potential amplitude (APA). The development of such beat-to-beat alternations is termed a bifurcation. It has been hypothesized that such bifurcations could promote temporal and spatial cardiac electrophysiological heterogeneity that in turn could facilitate the induction of
reentry and might be the cellular mechanism of drug-induced proarrhythmia.5,6

Bifurcations and irregular cardiac action potential properties including APA, APD, and cardiac excitability were also observed during regular pacing under conditions simulating various diseased states.5,7 These experimentally observed irregular cellular electrodynamics were also reproduced using computer simulation of realistic cardiac cell models in which the slope of the APD restitution curve was increased to specified levels.5-8 Although these isolated tissue studies have strongly suggested that such irregular cellular dynamics may be potential mechanism(s) for reentrant arrhythmias in the in situ heart,4,5 there exists no report at present that has shown the presence of irregular cellular dynamics in cardiac cells isolated from in situ arrhythmic ventricles. Furthermore, the presence of irregular cardiac cellular dynamics during drug-induced proarrhythmia also remains undefined. Consequently, the purpose of the present study was to determine whether ventricular myocardial cells isolated from in situ drug-induced arrhythmic ventricles manifest alternans and beat-to-beat irregular action potentials (bifurcation) during periodic stimulation. The results indicate that ventricular myocardial cells isolated from quinidine-induced arrhythmic ventricle do manifest bifurcations and irregular action potential dynamics that may predispose to ventricular tachyarrhythmias in vivo.

Methods
Surgical Preparation

Ten mongrel dogs were anesthetized with intravenous sodium pentobarbital (30–35 mg/kg), intubated with cuffed endotracheal tube, and ventilated with room air using a Harvard respirator at 4 cm H2O pressure. A Teflon catheter (i.d., 1.58; o.d., 3.17) was placed in the right carotid artery and advanced to the ascending aorta to monitor aortic blood pressure, and another catheter was inserted in the right jugular vein for systemic drug injections. A 6F USCI bipolar catheter was inserted through the left jugular vein and positioned at the right ventricular apex under fluoroscopic control for the purpose of pacing the ventricle.9 Surface lead ECG signals (I, III, and V1) and aortic blood pressure were continuously monitored; hard copy was obtained at 50–100 mm per second paper speed on a Honeywell VR-16 oscilloscopic-photographic recorder. In seven dogs, increasing cumulative doses of intravenous quinidine (quinidine gluconate injection USP, Eli Lilly) were administered for the purpose of inducing ventricular tachycardia and/or ventricular fibrillation (VT/VF).10 This model of systemic cardiac intoxication was used to ensure that individual cells subsequently selected for in vitro microelec-

trode studies would be reasonably representative (see below). Each dose of quinidine (10 mg/kg) was administered intravenously over a 2-minute period at 30-minute intervals until VT/VF was induced. Two minutes after each dose, the ventricle in each dog was paced for periods of 5–10 minutes at cycle lengths of 300–500 msec with constant current (2-msec duration at twice diastolic threshold) to increase rate-dependent myocardial uptake and subsequent toxicity of quinidine.11,12 In all seven treated dogs (after various forms of VT/VF were induced), the hearts were rapidly removed by severing the major vessels. Right ventricular endocardial tissue blocks (1–2 cm, <2 mm thick) were then excised and mounted in a tissue bath. Immediately before removal of the hearts, 10 ml of venous blood was withdrawn from the jugular vein in each dog for plasma quinidine assay by fluorescence polarization immunoassay.13

In Vitro Microelectrode Studies

The isolated tissues were mounted in a bath with the pinned endocardial surface upward and were superfused with Tyrode’s solution maintained at 36.5±0.5°C at pH 7.4±0.02. Three similarly prepared tissues were isolated from control nontreated dogs. The Tyrode’s solution was gassed with 95% oxygen and 5% nitrogen and had the following millimolar (mM) composition: NaCl 135, KCl 4.5, NaH2PO4 1.8, CaCl2 2.7, MgCl2 0.5, dextrose 5.5, NaHCO3 12 in triple-distilled deionized water.14 Both the tissue bath and the Tyrode’s stock solutions were continuously gassed with 95% oxygen and 5% nitrogen. The preparations were first regularly stimulated at 1,000-msec basic cycle length (BCL) with bipolar silver electrodes that were Teflon coated except at the tip (0.12-mm diameter).

Transmembrane action potentials were recorded with machine-pulled, glass capillary electrodes from the most superficial ventricular muscle cells (i.e., two to four cell layers deep) because the most superficial (one to two cell layers) are made of Purkinje cells.14,15 Unless otherwise specified, the stimulus intensity was twice diastolic current threshold with 2-msec duration and was applied within 1 mm of the microelectrode. The effects of different BCLs of stimulation (spanning 2,000–250 msec or until block was reached) and in some cases the effects of increasing stimulus current intensity on APD, APA, and excitation patterns were then evaluated. In four of the seven quinidine-treated tissues, two simultaneous action potentials from two different cells along with an extracellular bipolar electrograms (6USCI) were recorded from the endocardial surface. The two microelectrodes were placed close to the two poles of the bipolar electrode. The line connecting the stimulating electrode, the two microelectrodes, and the two poles of the bipolar was parallel to the long axis of the superficial endocardial fiber orientation. The distance between the stimulating electrode and the proximal recording microelectrode was <1 mm.

The protocol of the study consisted of recording sequentially from superficial ventricular muscle cells at multiple sites (seven to 14 cells in each tissue) during progressive increases in the frequency of stimulation. APD restitution curves, i.e., the relation of APD with respect to its previous diastolic interval (electrical restitution) of ventricular muscle cells both in control nontreated and in quinidine-treated preparations, were constructed with the extrastimulus method during regular drive at a BCL of 1,500 msec.16,17 During the studies in quinidine-intoxicated tissues, the Tyrode’s solution contained 10 µg/mL quinidine gluconate (Lilly) to maintain toxicity. Action potentials and bipolar electrograms were first recorded on an analog tape recorder (Bell & Howell, 4010 CRP) and then played back on a Honeywell (VR-16) for analysis.
TABLE 1. Steady-state Action Potential Properties of Ventricular Muscle Cells

<table>
<thead>
<tr>
<th>Group</th>
<th>RMP (mV)</th>
<th>APD_{00} (msec)</th>
<th>APA (mV)</th>
<th>V_{max} (V/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast depressed (n=37)</td>
<td>-70±3</td>
<td>392±21</td>
<td>79±7</td>
<td>65±22</td>
</tr>
<tr>
<td>Slow response (n=18)</td>
<td>-61±4</td>
<td>222±14</td>
<td>62±6</td>
<td>...</td>
</tr>
<tr>
<td>Nontreated control (n=16)</td>
<td>-84±3</td>
<td>204±12</td>
<td>123±7</td>
<td>201±19</td>
</tr>
</tbody>
</table>

RMP, resting membrane potential; APD, action potential duration for 100% repolarization; APA, action potential amplitude; V_{max}, maximum slope of phase-zero depolarization.

All three comparisons are significantly different from each other (p<0.01). Basic cycle length of stimulation was 1,500 msec.

**Statistical Analysis**

The paired t test was used to test differences in parameters between groups of cells. ANOVA was used for repeated measurements in a given group during the comparisons of the various parameters measured at different BCLs. A value of p<0.05 was considered significant.

**Results**

**Quinidine-Induced Ventricular Tachyarrhythmias in Intact In Situ Hearts**

Three of the seven quinidine-treated dogs developed VF, one spontaneously and two during pacing at 500-msec cycle length when the total cumulative dose of quinidine was 100 mg/kg. Two of the remaining five dogs developed runs of nonsustained and sustained (>30 seconds) VT when the total dose of quinidine was 80 mg/kg. In the remaining two dogs, a slow, wide QRS “agonal” rhythm (rate, 60 beats per minute) was induced when the total cumulative dose of quinidine was 100 mg/kg. The systolic aortic blood pressure during these terminal rhythms was 45±5 mm Hg and diastolic aortic blood pressure was 30±4 mm Hg. Quinidine plasma levels at the time of removal of the hearts ranged between 45 and 74 μg/mL, with a mean of 54±21 μg/mL (mean±SD). When ventricular tachyarrhythmias developed, the hearts were rapidly removed and placed in cold (4°C), oxygenated Tyrode’s solution for isolated in vitro microelectrode studies.

**Steady-state Action Potential Properties**

Both depressed fast-response (with resting membrane potential [RMP] between -75 mV and -66 mV) and slow-response (with RMP more positive than -65 mV) ventricular muscle cells were recorded from the superficial endocardial ventricular muscle cell layers. No ventricular muscle cell with RMP more negative than -79 mV could be recorded from the quinidine-toxic tissues. At relatively long cycle lengths of stimulation (>1,000 msec), each stimulus was followed by an action potential that had a fixed morphology in both fiber types. Table 1 describes action potential properties of the two quinidine-toxic cell types and the control nontreated groups of ventricular muscle cells.

No spontaneous diastolic depolarization or automatic activity either in the form of early or delayed afterdepolarizations could be observed or induced in any of the quinidine-intoxicated cells at all BCLs studied (2,000–300 msec).

**Dynamic Action Potential Properties**

**Alternans versus frequency of stimulation.** As the cycle length of stimulation was progressively decreased, the constant one-to-one stimulus response pattern (seen at relatively longer cycle lengths of stimulation) disappeared, and alternation of APD and APA emerged instead. These alternations depended on the frequency of stimulation and on the type of ventricular muscle cell studied (i.e., depressed fast-response versus slow-response fibers). Figure 1 illustrates the emergence of such alternans in a fast depressed cell as the stimulation frequency is increased. In 30 of 37 fast depressed fibers and in 11 of 18 slow-response cells, alternans of APD and APA emerged as the frequency of stimulation was

![Figure 1](http://circ.ahajournals.org/)

**FIGURE 1.** Comparative effects of progressive shortening of the cycle length (CL) of stimulation in normal (left panels) and quinidine-toxic (right panel) subendocardial ventricular muscle cells. A stable and constant action potential duration (APD) and action potential amplitude (APA) is present at each of the stimulating CLs tested (2,000–300 msec). In contrast, the cell isolated from a quinidine-intoxicated dog, alternans of both APD and APA occurred when the CL of stimulation decreased from 900 to 750 msec. Further shortening of the CL to 600 msec resulted in irregular APD and APA dynamics. The horizontal bar is 0.5 seconds for the normal recordings and 1 second for the quinidine recordings.
Table 2. Amplitude Alternations in Quinidine-Toxic Ventricular Muscle Cells

<table>
<thead>
<tr>
<th>Difference in APD&lt;sub&gt;90&lt;/sub&gt; (msec)</th>
<th>Difference in APA (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL (msec)</td>
<td>BCL (msec)</td>
</tr>
<tr>
<td>1,000</td>
<td>800</td>
</tr>
<tr>
<td>900</td>
<td>700</td>
</tr>
<tr>
<td>800</td>
<td>600</td>
</tr>
</tbody>
</table>

Increased. Table 2 summarizes these findings. A stable and steady RMP was maintained during these episodes of alternans in APD and APA. No alternation could be induced in any of the 18 control nontreated ventricular muscle cells at cycle lengths ranging between 2,000 and 250 msec (Figure 1).

To determine whether alternation of APD and APA results from individual cell action potentials and not from alternate activation (resulting from alternate conduction block), we recorded the overall tissue activation pattern by simultaneously recording two cellular action potentials along with an extracellular bipolar electrogram. Figure 2 illustrates such an experiment. The extracellular bipolar electrogram and the two cellular action potentials demonstrate simultaneous in-phase alternans of APD and APA at all three recording sites. This suggests that beat-to-beat variability results from intrinsic (rhythmic) variability of cellular transmembrane ionic currents rather than by alternate sequence of activation. Similar observations were made in five additional quinidine toxic tissues. The influence of the sequence of activation with tissue sizes similar to ours has yielded a maximum of 6–8-msec APD difference in canine tissue when the direction of propagation was switched from longitudinal to transverse direction.

In-phase patterns of alternation could, however, switch to out-of-phase (discordant) alternation patterns. In some experiments, two adjacent cells could manifest differing APD and/or APA properties during the same stimulus, creating spatial and temporal dispersion of action potential properties. Figure 3 illustrates one example of out-of-phase APD alternans. In-phase alternation of APD is seen initially but evolves over time to an out-of-phase pattern of alternation, thus creating temporal and spatial gradient of APD. Similar observations were also seen in three other quinidine-toxic tissues in which both APD and APA alternations became out of phase.

Alternans versus current strength of stimulation. It has been suggested that stimulus amplitude can modify the dynamic behavior of normal sheep cardiac Purkinje fiber action potentials during repetitive stimulation. Specifically, a critical stimulus amplitude, i.e., twice diastolic current threshold, was found to be necessary for the induction of alternans. Higher and lower current levels were often unable to induce alternans. In three quinidine-toxic ventricular muscle cells, we tested the effect of changes in stimulation current amplitude on action potential dynamics. Figure 4 illustrates one such experiment. When APD and APA alternation occurred at twice diastolic current threshold, an increase in the stimulus amplitude caused alternation to disappear. However, when the frequency of stimulation was further increased and stimulus amplitude was maintained at these elevated levels, alternans of APD and APA reappeared again (Figure 4). This change occurred without changing stimulus response latency, suggesting direct excitation by the stimulus at the proximal site. This supports the concept that alternans is caused by intrinsic cellular ionic alterations rather than by changes in the sequence of activation. Furthermore, these studies indicate that a critical stimulus amplitude, which depends on the frequency of stimulation, is needed for the induction of alternans. Because such a critical level of current amplitude (i.e., twice diastolic current threshold) is necessary to demonstrate irregular dynamics, we maintained the level of stimulus amplitude at twice threshold to detect potential irregular cellular dynamics in quinidine-toxic cells in subsequent studies.

Dynamics of Excitability and Frequency of Stimulation

It was shown in normal sheep cardiac Purkinje fibers and in aggregates of chick embryonic ventricles that as
the BCL of stimulation was decreased, there was a progressive and predictable decrease in the activation ratio. These patterns of activation were considered to be stable stimulus–response locking when the same sequence of action potentials and dropped beats was repeated several times, typically four to 10 times. In the present study, we observed stable stimulus–response locking patterns in eight fast depressed quinidine-toxic ventricular muscle cells. Figure 5 illustrates one such example. As the BCL of stimulation was decreased from 800 to 700 msec, the stimulus–response ratio changed from stable 1:1 locking (activation ratio, 1) to stable 3:2 locking (activation ratio, 0.66) after transient unstable locking patterns of 4:3 and 5:4. The 3:2 locking pattern could be promptly reversed to 1:1 locking upon lengthening of the BCL of stimulation from 700 msec back to 800 msec. Further decrease in the BCL of stimulation from 800 to 600 msec changed the locking from 1:1 to 2:1 (activation ratio, 0.50), and with further decrease of BCL...
of stimulation to 500 msec, a stable locking pattern of 1:0 emerged. Earlier studies in non–drug-treated cardiac cells demonstrated intermediate locking patterns (Devil’s staircase), which could be reasonably well predicted by Farey’s series (despite an inherent noise factor).5,20 Farey’s series predicts the order of stimulus–response patterns that occur as a result of a parameter change, including changes in the frequency of stimulation, changes in the stimulus amplitude, and time-dependent recovery processes.5,19 For example, one can predict through Farey’s series the phase-locking pattern between 1:1 and 3:2 by adding the numerators and the denominators of adjacent patterns and obtain in this particular example the 4:3 phase-locked pattern (i.e., 1+3=4 and 1+2=3). With similar procedures, one can easily compute the presence of 5:4 phase locking between two adjacent patterns of 1:1 and 4:3. These two locking patterns transiently occurred in our example given in Figure 5. Theoretically, there exists an infinite number of steps between two adjacent patterns of phase locking that even the Devil cannot climb (Devil’s staircase). However, it is questionable whether these theoretical patterns can be precisely duplicated in experimental settings because highly controlled progressive change in a parameter value may not be practical. More studies are needed to clarify this issue. It must be noted that in our quinidine-toxic cells, transient rather than stable locking patterns often emerged. This indicates that changes do occur constantly during a single specified condition despite the common assumption that the experimental conditions are “controlled.” For example, the concentration of sodium channel–bound quinidine may continuously change because it is known that quinidine exhibits frequency-dependent block of sodium channels.11

In addition to frequency-dependent locking patterns, we found that quinidine-toxic ventricular muscle cells also manifested time-dependent locking patterns, when the BCL of stimulation remains constant. To separate the frequency and time dependency, in five quinidine-toxic cells the effect of time on the pattern of phase locking was evaluated while maintaining constant frequency. Figure 6 illustrates one such experiment. As stimulation frequency was maintained constant, the 1:1 locking evolved to 2:1, then 3:1, and finally to 4:1. With further decreases in the BCL of stimulation to 720 msec, phase locking of 1:0 occurred. In three preparations, simultaneous recording of two cells showed that locking could occur either in or out of phase, and in-phase locking could switch over time to out-of-phase locking. These findings demonstrate that changes in either the duration or the rate of periodic stimulation can cause the observed locking patterns. This may explain at least in part the unexpected and transient unstable locking patterns observed in our quinidine-toxic ventricular muscle cells. Time-dependent changes in the coupling pattern have been observed in embryonic chick heart cell aggregates during periodic stimulation.21 Similarly, incorporation of time-dependent parameters in a model describing the complex dynamics of atrioventricular conduction reflecting recovery of excitability successfully predicted complex patterns of atrioventricular Wenckebach patterns of conduction in humans.22

In normal nontreated ventricular muscle cells, repetitive stimulation at a BCL of 1,000–300 msec (or until block) induced no locking pattern. The 1:1 response pattern was observed in all ventricular muscle cells (n=8) studied until failure of capture occurred.

Frequency of Stimulation and Aperiodic Cellular Dynamics

At a critical BCL of stimulation, quinidine-toxic cells generated action potentials that had irregular beat-to-beat APA and APD dynamics (Figure 1 and Figure 7). In 20 of 37 depressed fast-response fibers, irregular action potential dynamics were induced at a mean cycle length of stimulation of 625±250 msec (range, 850–280 msec). During the irregular activity, no periodic pattern in either APD or APA could be detected for up to 200 consecutive beats. We could see neither the dynamic phenomenon of “recurrent fluctuations” between regular and irregular activity nor the behavior of “multiphysiological" with locking previously reported in Purkinje fibers.5 This type of irregular activity that is seen at fixed cycle lengths of stimulation and at a constant
stimulus amplitude is characterized by random fluctuations in stimulus response patterns that have common activation ratios (i.e., 2:1, 6:3, 18:6, etc.)². It has been suggested that multirhythmicit has been related to supernormal excitability in cardiac Purkinje fibers. However, its relevance to other forms of action potential dynamics and eventually reentrant arrhythmias remain undefined at the present. In slow-response fibers however, no aperiodic or irregular action potential dynamics could be observed in any of the 18 slow-response ventricular muscle cells studied. Instead, progressive increases in the rate of stimulation caused various patterns of phase locking to emerge that ultimately led to inexcitability.

We did not observe irregular action potential dynamics in the 18 normal nontreated ventricular muscle cells during regular pacing at cycle lengths of 2,000–280 msec. However, the rate-dependent shortening of APD observed in ventricular muscle cells was regularly observed in all the normal cells studied (Figure 1).

Irregular Action Potential Dynamics and Induction of Nondriven Responses

It has been suggested that asynchronous firing and repolarization of cells or groups of cells may generate local excitatory currents that can make reexcitation possible. In two quinidine-toxic preparations, nondriven action potential–like responses were induced during phase three repolarization when the BCL of stimulation reached a critical level within which irregular dynamics are expected to occur. Figure 8 illustrates one such example: The two sampled cells in this case were separated by about 1 cm and showed only a minimal alternans in their APD and APA as the BCL of stimulation was decreased from 600 to 400 msec. The rapid stimulation and resulting asynchrony induced nondriven single responses that were not sustained (they subsided when the stimulation was turned off). These findings are suggestive of a local reexcitation phenomenon caused by the mechanism of reflection seen in Purkinje fibers.²³

Action Potential Duration Restitution Curves

It has been suggested that an increase in the slope of the APD restitution curve may be important in the induction of APD alternans and aperiodic APD dynamics during regular stimulation at a critical frequent-
nisms may be involved in the genesis of rate-dependent APD dynamics.

Discussion

Our results demonstrate that antiarrhythmic drug toxicity increases beat-to-beat variability in APD and APA during periodic stimulation and therefore causes temporal and spatial nonuniformities in action potentials. These observations are comparable with earlier experimental reports using cardiac tissue exposed to stressful stimuli and are consistent with behavior seen in other nonlinear dynamical systems subjected to stress.

The irregular behavior induced by quinidine adds further experimental evidence that cardiac cells behave as "nonlinear dynamical systems." The action potential dynamics induced by quinidine closely resemble the dynamics observed in cardiac cells exposed to a variety of stressful conditions including low temperature, the electrical uncoupler heptanol, and rapid pacing.

Arrhythmic Consequences of Cellular Bifurcation Dynamics

The irregular action potential dynamics of quinidine-toxic cells could create spatial and temporal inhomogeneities in repolarization and conduction in the ventricles. The overall similarity in BCL at which arrhythmias developed in vivo (BCL, 500 msec) and the BCLs at which irregular APD dynamics occurred in vitro (average BCL, 625 msec) suggests a probable link between the occurrence of irregular APD dynamics and arrhythmias in the intact heart. These irregular APD dynamics may facilitate reentry either by the mechanism of reflection or by the mechanism of circus movement resulting from inhomogeneity in conduction. Such a possibility is suggested by our ability to induce nondriven activity in the isolated tissue during critically rapid BCL of stimulation. At present, we do not know if a similar cellular mechanism is also operative in the intact ventricle or whether or not pacing-induced VT/VF in the quinidine-intoxicated dog resulted from such cellular dynamics.
nondriven beats could have been caused by an automatic mechanism. Similar findings were also reported by others. Furthermore, Wit and associates have shown that high concentration of quinidine (>10 μg/mL) can block triggered activity in canine coronary sinus fibers. It has been shown in canine studies that dispersion of repolarization caused mainly by dispersion of monophasic APD by dispersion of refractory periods could initiate reentrant rhythms in the intact ventricle. In fact, mapping of ventricular activation sequences has shown that sustained rhythmic activity could be induced by reentry both in chronically infarcted intact ventricular myocardium and in isolated cardiac tissue by a mechanism dependent on dispersion of repolarization, nonuniform recovery of excitability, and inhomogeneity of conduction. Dispersion of repolarization could also be induced by some antiarrhythmic drugs and could explain their proarrhythmic potential. The asynchronous firing, phase-locking patterns, and irregular action potential dynamics that we have demonstrated in the present study during incremental stimulation can create temporal and spatial heterogeneity (“dynamic dispersion”) that is highly suitable for reexcitation by circus movement reentry or by the phenomenon of reflection. It is worth mentioning that such dynamic nonuniformity dissipates at relatively longer BCLs of stimulation and manifests itself only during rapid rates of stimulation.

Alternative mechanisms may also be operative for the induction of reentry in the quinidine-toxic ventricles. Conduction slowing and conduction block may also be caused by increased cell-to-cell coupling resistance and by the presence of slow-response fibers. These fibers could be activated reflexly by increased background sympathetic tone as a result of hypotension induced by toxic concentrations of quinidine. These potential mechanisms may be operative either alone or in concert with our proposed novel mechanism of dynamic nonuniformity (i.e., dispersion) in causing reentry in the intact ventricle.

**Cellular Mechanisms of Unstable Action Potential Dynamics**

In an attempt to determine the possible cellular mechanisms of APD alternans, we constructed APD restitution curves. It has been suggested that increasing the steepness of the slope of the APD restitution curve could lead to APD alternans and to irregular APD dynamics. In the present study, we found an increased slope of the APD restitution curve of quinidine-toxic cells compared with control nontreated cells. Iteration of the equation describing the APD restitution curve showed that increases in the steepness of the slope (>1) was associated with bifurcation and irregular APD dynamics.

Alternans in the numerical model occurred at faster rates of stimulation compared with the rate at which the quinidine-toxic muscle cells showed alternans. This indicates that in addition to the slope, other factors may also be operative in the genesis of APD bifurcations and irregular APD dynamics. This also explains at least in part why relatively modest increases in the slope (i.e., final values <1) could also cause alternans at a critically high frequency of stimulation.

**TABLE 3. Values of the Constants of the Biexponential Equation Used for Iteration**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Quinidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>303.98</td>
<td>666.24</td>
</tr>
<tr>
<td>B₁</td>
<td>2,039.47</td>
<td>5,449.37</td>
</tr>
<tr>
<td>γ₁</td>
<td>36.43</td>
<td>45.95</td>
</tr>
<tr>
<td>B₂</td>
<td>89.03</td>
<td>398.99</td>
</tr>
<tr>
<td>γ₂</td>
<td>724.86</td>
<td>268.01</td>
</tr>
</tbody>
</table>
The ionic mechanism(s) of quinidine-induced increase in the slope of APD restitution remain undefined. Voltage-clamp studies on isolated guinea pig ventricular myocytes have shown that quinidine delayed the activation of the delayed rectifier outward current (I_K) and reduced its amplitude. Such an effect could conceivably cause an upward shift in the APD restitution curve with an attendant increase in its slope. In a recent computer simulation of two-dimensional impulse propagation, an isolated increase in the slope of the APD restitution facilitated the induction of APD alternans and the induction of nonstationary double spiral waves. Similar spiral waves have been induced in thin sheets of sheep epicardial muscle cells during appropriate stimulation protocols. It has been suggested that APD alternans during acute ischemia could result from alternation of intracellular calcium ([Ca]_i) transients and that such APD alternation could promote the initiation of spontaneous VF in the intact ventricle. An important regulatory role of [Ca], on the APD restitution in canine endocardial ventricular muscle cells has recently been emphasized.

The ionic mechanism of altered dynamics of APD and rate-dependent block of excitation during rapid stimulation in quinidine toxic cells remains undefined. Delmar et al have shown in isolated guinea pig ventricular myocytes that the time course of I_K deactivation kinetics was similar to the time course of growth of subthreshold depolarizing responses. Such a property of ventricular myocytes was shown to cause various patterns of rate-dependent excitation failure (phase locking). These mechanistic suggestions were confirmed by computer simulation by the same authors. More recently, Joyner et al have shown that delayed recovery of excitability of ventricular cells could lead to variable degrees of excitation block during a sudden increase in the rate of stimulation. These authors suggested that beat-to-beat accumulation of inactivation can lead to variable degrees of activation ratios. We do not know if quinidine can delay the deactivation kinetics of I_K in canine ventricular muscle cells as it does in rabbit nodal cells or delay recovery of excitability, or both.

Simulation studies using modified Beeler-Reuter cardiac cell models have shown that rate-dependent excitation failure and irregular APA dynamics during regular stimulation could also result from delayed recovery of the sodium current and from I-type calcium currents. We do not know which of these potential ionic mechanisms may be responsible for the induction of these observed dynamic behaviors because quinidine could interact simultaneously with multiple ionic channels and potentially manifest nonspecific ion channel block at high (toxic) concentrations.

Clinical Relevance

The tachyarrhythmias that occur during acute ischemia are often preceded by depolarization and repolarization alternans. One study has shown an increased likelihood of VF occurrence during discordant (out-of-phase) repolarization alternans. More recently, abnormal monophasic APD (MAPD) restitution curves resulting from exaggerated APD shortening were observed in patients with ventricular disease and complicating VT. The MAPD restitution curves were altered in diseased areas in these patients, often showing greater steepness compared with curves obtained from normal zones. The greater steepness of APD restitution curves in these patients could cause greater temporal and spatial dispersion of repolarization leading to VT. Therefore, MAPD restitution curves may allow monitoring antiarrhythmic drug toxicity (proarrhythmia) in humans since MAPD restitution curves with the contact electrode closely resemble transmembrane cellular APD restitution.

Summary

We have shown that ventricular muscle cells isolated from quinidine-induced arrhythmic canine ventricles manifest rate-dependent beat-to-beat changes in APD and APA including phase locking (alternans) and irregular dynamics. The cellular mechanism of the dynamic APD changes could be analytically described by quinidine-induced increase of the slope of the restitution curve, whereas the mechanism of quinidine-induced APA dynamics remains undefined.
These dynamic cellular spatiotemporal nonuniformities that can be explained with a reasonable degree of certainty using arguments based on nonlinear dynamics provide new insights into the origin of drug-induced proarrhythmia and offer a novel cellular dynamic mechanism for sudden cardiac death caused by VT/VF.

Acknowledgments

We thank Avile McCullen for his excellent technical assistance, Sergio Farber, PhD, of the Department of Clinical Chemistry at Cedars-Sinai, for assaying plasma quinidine levels, Michael R. Guevara, PhD, for referring us to Reference 8, and Masamichi Gotoh, MD, for useful discussion.

References

1. Lewis T: Notes upon alternation of the heart. Q J Med 1911;4:141–145


Action potential alternans and irregular dynamics in quinidine-intoxicated ventricular muscle cells. Implications for ventricular proarrhythmia.
H S Karagueuzian, S S Khan, K Hong, Y Kobayashi, T Denton, W J Mandel and G A Diamond

Circulation. 1993;87:1661-1672
doi: 10.1161/01.CIR.87.5.1661
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/87/5/1661

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at: http://circ.ahajournals.org/subscriptions/