Modification of the frequency- and voltage-dependent effects of quinidine when administered in combination with tocainide in canine Purkinje fibers

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ABSTRACT  Frequency- and voltage-dependent modification of drug-induced inhibition of maximal upstroke velocity of the action potential (Vmax) by the combined administration of two class I antiarrhythmic drugs was studied in canine Purkinje fibers, taking depression of upstroke velocity as an indicator of sodium channel blockade. The kinetics of onset of drug-induced Vmax depression and the time course of Vmax recovery were studied after exposure to therapeutic concentrations of tocainide (50 μM) and quinidine (5 μM) both singly and in combination. The rate constant for onset of block during a drive train at a cycle length of 600 msec was 0.95 ± 0.32 pulses in the presence of tocainide and 5.61 ± 0.50 pulses in the presence of quinidine. The magnitude of block was three times greater with quinidine than with tocainide. The magnitude of block produced by the combination was no greater than that produced by quinidine alone and may be partly due to abbreviation of action potential duration by tocainide. Onset of block in the presence of the combination was best fitted by a double exponential with rate constants of 0.88 ± 0.19 and 6.47 ± 1.36 pulses. Vmax recovery after termination of a rapid train of impulses was delayed by both drugs. Poststimulation recovery from either tocainide- or quinidine-induced block was characterized by a single time constant (1.04 ± 0.49 and 4.81 ± 0.76 sec, respectively), while that of the combination was characterized by two time constants (0.43 ± 0.22 and 5.94 ± 0.56 sec), presumably corresponding to dissociation of each drug from the sodium channel receptor. The mixture of the two drugs produced a large depression of Vmax of early diastolic premature responses without producing much further depression of Vmax than that produced by quinidine alone at longer coupling intervals. The time constant of recovery from tocainide-induced block was greatly dependent upon membrane potential. After steady-state changes in frequency, the combination produced a greater depression of Vmax at rapid heart rates compared with that produced by quinidine alone, but abbreviated action potential duration more at slower heart rates. Addition of tocainide to fibers equilibrated with quinidine shifted the Vmax–membrane potential relationship to more hyperpolarized potentials, resulting in greater depression of Vmax at more depolarized membrane potentials with little or no additional depression of Vmax at more negative membrane potentials. The results provide a rationale for a possible enhanced antiarrhythmic efficacy of a combination of two class I drugs that have different kinetics of interaction with the sodium channel.


A DECREASE in the maximal upstroke velocity of the action potential (Vmax) leading to a decrease in conduction velocity is characteristic of the action of class I antiarrhythmic drugs. It is now recognized that these drugs have fairly complex effects on myocardial conduction via their modulated effects on sodium channels.1–3 Sodium-channel blockade by class I antiarrhythmic drugs is modulated by the rate of use of the channel. Rapid trains of action potentials or depolarizing pulses will enhance channel block above the basal (resting) level. After such conditioning trains channel block relaxes back to the basal level with widely differing time constants. This rate of unblocking, which is dependent on drug structure and its associated molecular weight and lipid solubility, helps determine what action potential frequencies an excitable cell can follow during drug treatment.4,5 Drugs of low molecular weight and high lipid solubility (e.g., lidocaine) tend to have rapid rates of unblocking.4,6 Such drugs would tend to show relative selectivity for depressing early
premature impulses or those elicited at rapid rates. Drugs with slower rates of unblocking (e.g., quinidine) would tend to be more nonselective in their depressant effects over a relatively wide range of stimulation frequencies or premature intervals. Hondeghem and Katzung have suggested that a combination of two class I agents with different rates of unblocking, such as lidocaine and quinidine, might provide a more effective depression of early premature impulses or those elicited at rapid heart rates than either drug alone while not depressing conduction at normal heart rates beyond that produced by the drug with slower kinetics. Such a combination may be desirable for the treatment of reentrant arrhythmias. In fact, Duff et al. showed that a combination of mexiletine and quinidine was more effective against ventricular arrhythmias than either drug alone.

Tocainide is an orally active lidocaine congener. If this drug interacts with the sodium channel in a manner similar to that of lidocaine, then a combination of tocainide and quinidine might be a rational approach for the long-term control of reentrant ventricular arrhythmias. Furthermore, tocainide might also be beneficial in preventing some of the toxic manifestations associated with quinidine therapy by preventing quinidine-induced prolongation of repolarization. To gain further insight into the interaction between these two drugs, we investigated the kinetics of onset of drug-induced development of block during the action potential and the time course of recovery from block during diastole in canine Purkinje fibers exposed to therapeutic concentrations of tocainide and quinidine both singly and in combination. The dependence of sodium-channel block on stimulation rate and diastolic membrane potential was also determined.

Methods

Mongrel dogs of both sexes were anesthetized with sodium pentobarbital (30 mg/kg) and their hearts were removed via a left thoracotomy. Both ventricles were thoroughly flushed with chilled, oxygenated Tyrode’s solution. Purkinje fibers with small pieces of attached ventricular muscle were quickly excised and pinned to a Sylgard block at the bottom of a tissue bath (8 ml capacity) and continuously perfused at a rate of 10 to 12 ml/min with modified Tyrode’s solution aerated with 95% O2-5% CO2. Our standard Tyrode’s solution contained (in mM): NaCl, 119.0; KCl, 4.0; CaCl2, 1.8; MgCl2, 0.5; NaHPO4, 0.9; dextrose, 5.5; and NaHCO3, 25. pH of the solution was 7.33 ± 0.03. The temperature was maintained at 37 ± 0.2°C.

We used small, free-running intertrabecular Purkinje fiber bundles (length < 5 mm) with attached ventricular muscle. These preparations displayed minimal diastolic depolarization and provided stable membrane potentials more negative than −80 mV during prolonged periods of quiescence. Preparations that displayed automaticity were not used.

The preparations were stimulated with rectangular pulses through bipolar tungsten electrodes etched in sodium nitrate and coated with Formvar. Electrical stimulation was provided by a Model RS-660 Timing Simulator/Word Generator controlled by an HP9816 computer in combination with a Digitimer stimulus isolation unit (model DS2). To reduce errors in Vmax measurements the recording and stimulating electrodes were positioned such that upstroke velocities did not change more than 5% with changes in stimulus strength.

Transmembrane potentials were recorded with glass microelectrodes filled with 3M KCl and coupled to the input of a high-impedance, capacitance-neutralized amplifier (Model KS-700, WPI instruments). The output of the KS-700 was displayed on a Tektronix 5113 dual-beam storage oscilloscope and simultaneously displayed in digital form on a Data 6000 waveform analyzer (Data Precision, Inc.).

During control measurements and during equilibration with drug or a combination of drugs, the preparations were stimulated at a basic cycle length of 1000 msec. Action potential variables were recorded on-line every 30 sec until steady-state effects were observed.

The influence of drugs on action potential variables as a function of steady-state changes in stimulation frequency was examined by varying the cycle length from 300 to 2000 msec. Sufficient time (4 min or more) was allowed at each cycle length to permit Vmax to reach a new steady-state value.

To study the rate of development of block with drive, the preparations were driven by 45 beat trains at an interstimulus interval of 600 msec. Rest periods between trains of stimuli were long enough to ensure full recovery from rate-dependent effects. Rate of decline of Vmax during the stimulus train and the magnitude of rate-dependent block are dependent on the interstimulus interval within the train. The magnitude of block was greater and the rate of onset of block was faster with decreases in the interstimulus interval with both tocainide and quinidine. This reflects the well-known use dependence of drug action. We chose an interstimulus interval of 600 msec because, in the presence of quinidine, the first action potential of the train is so prolonged (see figure 5) that if we had used shorter interstimulus intervals, the second action potential of the train would have occurred during the repolarization phase of the first response, resulting in voltage dependent depression of Vmax. Thus, in all preparations, an interstimulus interval of 600 msec was used so that comparisons between drugs and the combination could be made.

To determine the recovery of Vmax from block, test pulses were introduced at varying diastolic intervals after a stimulus train had produced a steady level of block. The onset of diastole was defined as that time when repolarization had returned to the maximum diastolic potential. After each test pulse, there was a 20 sec quiescent interval followed by another stimulus train. Test pulses were initially introduced during phase 3 of the last action potential of the train and then progressively later in the cycle. Initially, the test intervals were increased in small steps and then in progressively larger steps. The steps ranged from 10 to 5000 msec. Each recovery curve comprised at least 50 points.

To study drug effect on steady-state sodium inactivation, preparations were stimulated at cycle lengths of 5 sec under control conditions and 10 to 20 sec in the presence of drugs. Cycle lengths longer than 20 sec could not be studied because of spontaneous activity. The fibers were then exposed to progressively higher potassium concentrations in 1 to 2 mM steps until the preparations stopped responding. Each potassium concentration was applied until steady-state effects on Vmax were observed. The preparations were then returned to a 2.7 mM potassium Tyrode’s solution and exposed to a second intervention and the potassium concentrations were again increased in 1
to 2 mM steps as before. During alterations of the potassium concentration, Vmax and membrane potential were recorded on-line every 5 sec.

Tocainide used was tocainide hydrochloride generously supplied by Astra Pharmaceuticals. Quinidine used was quinidine sulfate dihydrate obtained from J. T. Baker Chemicals. The drugs were added to Tyrode's solution made up from refrigerated stock solutions. Concentrations refer to the concentrations of the base. Therapeutic concentrations of both drugs were used, 5 \mu M (1.6 \mu g/ml) of quinidine and 50 \mu M (9.6 \mu g/ml) of tocainide. The concentration of quinidine that we have used is lower than that used in most previous studies in vitro. Plasma quinidine concentrations in patients receiving therapeutic doses have been reported recently to range between 1 and 7 \mu g/ml.\textsuperscript{10,11} Since the drug is 70% to 80% protein bound the free drug concentration in plasma is only 20% to 30% of that measured by conventional assay techniques. Therefore, when the drug is studied in the tissue bath with the use of protein-free superfusates, concentration of drug should be in the range of 0.2 to 2 \mu g/ml. The concentration of quinidine that we have chosen is within this range. Similar concentrations have been studied by Shen and Antzelevitch.\textsuperscript{12} Therapeutic plasma concentrations of tocainide vary from 4 to 23 \mu g/ml.\textsuperscript{13,14} Since the drug can be as little as 20% protein bound, the concentration we have chosen is again within the therapeutic range.

It was necessary to expose the preparations to quinidine for at least 1 hr before a steady-state effect on action potential characteristics was obtained. In the presence of tocainide, steady-state effects were usually achieved within 20 min. Thus, in the majority of experiments, onset of and recovery from block in the presence of the combination were compared with those in quinidine alone since it was not possible to keep single impalements long enough to study both drugs and the combination in the same cell.

**Data analysis.** All action potential variables were determined with a Data 600 waveform analyzer. This instrument consists of a 68,000 microprocessor with a 14-bit analog-to-digital converter that can acquire data at a rate of up to 100 kHz with a resolution of 0.06 mV. The action potential waveform was digitized at two different rates. The action potential waveform at the fastest digitizing rate, which was always set at 20 \mu sec, was used to determine all phase 0 variables. The waveform at the slower digitizing rate, which ranged from 0.6 to 1 msec, was used to determine action potential duration. Take-off potential, maximum diastolic potential, action potential amplitude (AMP), Vmax, membrane potential at which Vmax occurred (V), and action potential duration (APD) at the 50%, 75%, and 95% levels of repolarization were measured simultaneously on-line. By measuring both take-off and maximum diastolic potentials, we were able to detect the extent of any diastolic depolarization that may have occurred. In some experiments, we measured activation time, i.e., the time from the beginning of the stimulus pulse to Vmax.

Where applicable, data are expressed as mean \pm SD. Student's t test was used to make statistical comparisons between drug effects. The time course of onset or recovery from block was defined with the use of a least square error nonlinear exponential fitting program (Hewlett-Packard Statistical Library). The time constant of Vmax block or recovery contained either one or two distinct components. Least squares analysis of the data indicated that each component (if more than one were present) could be approximated by a single exponential function of the form Y = A exp (- t / \tau) + B. No attempt was made to fit the data with more complex functions. However, when appropriate, it was determined whether double exponential functions fit the data better than single exponential functions. A double exponential was considered a better fit if the residual sum of squares for the double exponential was one-third or less than that for the single exponential.

Although we have taken all necessary precautions in measuring Vmax, interpretation of our results necessarily depends on the accuracy with which Vmax serves as an indication of the fast inward sodium current, an issue that is a matter of current controversy.\textsuperscript{15-17} For the purposes of the present study, Vmax only needs to be a qualitatively accurate indicator of peak inward sodium conductance so that a decrease in Vmax reflects a decrease in peak sodium current.

**Results**

**Effects on action potential characteristics.** Table 1 summarizes the effects of 50 \mu M tocainide, 5 \mu M quinidine, and their combination on action potential charac-

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>TOP</th>
<th>AMP</th>
<th>Vmax</th>
<th>APD 50%</th>
<th>APD 75%</th>
<th>APD 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TOP (mV)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>90.7 ± 4.4</td>
<td>123.0 ± 4.1</td>
<td>580 ± 123</td>
<td>226 ± 61</td>
<td>271 ± 65</td>
<td>312 ± 70</td>
</tr>
<tr>
<td>Tocainide</td>
<td>90.2 ± 4.6</td>
<td>121.7 ± 4.4</td>
<td>550 ± 123 \textsuperscript{A}</td>
<td>168 ± 48 \textsuperscript{A}</td>
<td>221 ± 52 \textsuperscript{A}</td>
<td>265 ± 56 \textsuperscript{A}</td>
</tr>
<tr>
<td>% change (%)</td>
<td>-0.8 ± 1.8</td>
<td>-1.4 ± 1.2</td>
<td>-5 ± 4 \textsuperscript{A}</td>
<td>-24 ± 8 \textsuperscript{A}</td>
<td>-18 ± 6 \textsuperscript{A}</td>
<td>-15 ± 5 \textsuperscript{A}</td>
</tr>
<tr>
<td>(n = 19)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>89.7 ± 2.4</td>
<td>127.4 ± 2.9</td>
<td>803 ± 131</td>
<td>238 ± 33</td>
<td>280 ± 32</td>
<td>323 ± 30</td>
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<tr>
<td>Quinidine</td>
<td>89.0 ± 3.5</td>
<td>123.6 ± 4.8 \textsuperscript{A}</td>
<td>692 ± 131 \textsuperscript{A}</td>
<td>200 ± 29 \textsuperscript{A}</td>
<td>277 ± 31</td>
<td>334 ± 27 \textsuperscript{A}</td>
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<tr>
<td>% change (%)</td>
<td>-0.8 ± 2.9</td>
<td>-2.9 ± 2.9 \textsuperscript{A}</td>
<td>-14 ± 6 \textsuperscript{A}</td>
<td>-16 ± 7 \textsuperscript{A}</td>
<td>-1 ± 5</td>
<td>+4 ± 6 \textsuperscript{A}</td>
</tr>
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<td>(n = 13)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Quinidine</td>
<td>89.6 ± 3.1</td>
<td>122.8 ± 4.0</td>
<td>630 ± 138</td>
<td>189 ± 20</td>
<td>274 ± 18</td>
<td>340 ± 23</td>
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<tr>
<td>Combination</td>
<td>88.7 ± 2.8</td>
<td>121.3 ± 4.6</td>
<td>604 ± 145 \textsuperscript{A}</td>
<td>160 ± 17 \textsuperscript{A}</td>
<td>246 ± 9 \textsuperscript{A}</td>
<td>310 ± 13 \textsuperscript{A}</td>
</tr>
<tr>
<td>% change (%)</td>
<td>0.9 ± 3.4</td>
<td>-1.3 ± 1.9</td>
<td>-5 ± 4 \textsuperscript{A}</td>
<td>-15 ± 6 \textsuperscript{A}</td>
<td>-10 ± 5 \textsuperscript{A}</td>
<td>-8 ± 4 \textsuperscript{A}</td>
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<td>(n = 9)</td>
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Values are mean ± SD. Fibers stimulated at a basic cycle length of 1000 msec.

**TOP =** take-off potential; **AMP =** amplitude; **APD 50% , APD 75% , APD 95% =** action potential duration at 50%, 75%, and 95% levels of repolarization.

\textsuperscript{A}p < .05.
teristics in Purkinje fibers stimulated at a basic cycle length of 1000 msec. Neither drug altered the maximum diastolic potential. Quinidine consistently depressed AMP and Vmax. Effects of tocainide on Vmax ranged from little or no depression to approximately 10% depression (average of 5%). Tocainide abbreviated all phases of the action potential while quinidine abbreviated the plateau but lengthened the terminal phase of repolarization. Addition of tocainide to fibers equilibrated with quinidine caused a small but significant further depression of Vmax, and abbreviated all phases of the action potential. 

Comparison of the effects of quinidine versus the combination on action potential characteristics during steady state changes in cycle length.

Effects on phase 0 characteristics. Quinidine progressively reduced the steady state level of Vmax in a rate-dependent manner as the basic cycle length was decreased from 2000 to 300 msec (figure 1). Addition of tocainide to the quinidine superfusate produced a further net decrease of Vmax (more block) at all stimulation rates. However, the additional decrease in Vmax was much greater at a cycle length of 300 msec than at a cycle length of 2000 msec. Similarly, there was much more further depression of amplitude at the shortest basic cycle lengths after addition of tocainide. In the presence of quinidine as well as the combination there was progressive hyperpolarization with decreases in cycle length. Similar results were obtained in two other experiments.

Effects on APD. Addition of tocainide to the quinidine superfusate abbreviated APD at all cycle lengths and at all levels of repolarization. However, the amount of shortening was much greater at the longest cycle lengths (figure 2). Similar results were obtained in two other experiments.

Comparison of the effects of tocainide, quinidine, and their combination on the rate of development and magnitude of frequency-dependent block. There was no frequency-dependent alteration in Vmax under control conditions. The magnitude and rate of development of block for each drug and the combination are summarized in table 2. The rate of onset of block in the presence of tocainide was very fast. In preparations with maximum diastolic potentials greater than −90 mV, steady state block developed within one or two action potentials and the magnitude of block was small. Figure 3 shows a typical example. The magnitude of rate-dependent block ranged from 1.29% to 15.0% in individual preparations. The first action potential of the stimulus train is an indication of the magnitude of block after a long rest period (tonic block). Tocainide either produced no tonic block or as much as 10% tonic block in individual preparations. This variability may be related to variable degrees of depolarization during the quiescent interval.

FIGURE 1. Typical example of the effects of steady state changes in basic cycle length upon take-off potential (TOP; top left), action potential amplitude (top right), and Vmax (bottom) in the presence of 5 μM quinidine, and a combination of 5 μM quinidine and 50 μM tocainide. Both the actual and normalized values of Vmax are plotted. Bottom left. The percentage values represent the additional depression of Vmax at each cycle length in the presence of the combination versus quinidine alone. The amount of depression at short cycle lengths is probably underestimated because of the rate-related changes in TOP. The cycle length was varied between 2000 and 300 msec. Each cycle length was maintained until steady-state effects were obtained beginning with the slowest cycle length. In this and all subsequent figures, quinidine is represented by triangles, the combination by squares.
The rate of onset of block in the presence of quinidine alone was much slower when compared with that in tocainide, while the magnitude of block was greater (table 2). This concentration of quinidine produces no membrane depolarization, only a small amount of tonic block, but marked use-dependent block in fully polarized tissue.

Figure 4 compares the rate of development of block for quinidine alone versus a combination of quinidine and tocainide in a preparation in which it was possible to maintain impalement during control conditions and after exposure to the drugs. It was necessary to expose the preparation to quinidine for at least 1 hr before a steady-state level of block was attained. Quinidine alone appeared to produce a small amount of tonic block but a much greater amount of rate-dependent block. Decline of Vmax during exposure to quinidine followed a single exponential. When the two drugs were added together, the amount of tonic block increased, but the magnitude of rate-dependent block was similar to that with quinidine alone. In all experiments the magnitude of rate dependent block with the combination was not statistically different from that of quinidine alone. The amount of tonic block with the combination was highly variable, ranging from no block to about 8% block. Since there were slight variations in the degree of depolarization during each quiescent interval, no conclusions can be made about the degree of tonic block. Decline of Vmax during exposure to the combination followed a double exponential. The first rate constant was similar to that obtained with tocainide alone in other preparations, while the second one was similar to that obtained with quinidine alone (table 2). After reexposure to quinidine alone, the curve obtained was similar to that after initial exposure to quinidine. Experiments were also conducted with 10 μM of quinidine alone and in the combination. The results were qualitatively similar.

**Rate-dependent effects on APD.** In all preparations, APD was also monitored during the stimulus train.

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**TABLE 2**

Comparison of the effects of tocainide, quinidine and their combination on the magnitude and kinetics of onset of rate-dependent block

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rate constant (pulses)</th>
<th>%RDB</th>
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<tbody>
<tr>
<td>Tocainide, 50 μM (n = 16)</td>
<td>0.95 ± 0.32&lt;sup&gt;A&lt;/sup&gt;</td>
<td>5.32 ± 3.40&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Quinidine, 5 μM (n = 15)</td>
<td>5.61 ± 0.50</td>
<td>15.81 ± 3.68</td>
</tr>
<tr>
<td>Tocainide, 50 μM, plus quinidine, 5 μM&lt;sup&gt;B&lt;/sup&gt; (n = 10)</td>
<td>0.88 ± 0.19&lt;sup&gt;A&lt;/sup&gt;</td>
<td>15.64 ± 4.87</td>
</tr>
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</table>

Values are expressed as mean ± SD.
RDB = Rate dependent block
<sup>A</sup>Significantly different from quinidine alone (p < .05).
<sup>B</sup>Onset of block in the presence of the combination was best fitted by a double exponential.
Typical drug effects on APD are shown in figure 5. Both under control conditions and in the presence of drugs, the first action potential of the train (preceded by a quiescent interval of 30 sec) was prolonged. Shortening during the train followed a biexponential decline. In the presence of quinidine the first action potential of the train was tremendously prolonged when compared with the control action potential, but shortened to below control values by the end of the train. The marked prolongation of the first action potential by quinidine was likely due to an effect on potassium channels and suggests that quinidine is able to block potassium channels in fully polarized tissue in the absence of stimulation. This is in contrast to the small degree of block of sodium channels in the absence of stimulation (figure 4). When tocainide was added to the preparation equilibrated with quinidine, the duration of all action potentials in the train shortened. However, a striking effect was the very much greater shortening of the first action potential. Shortening of all action potentials in the train would be accompanied by a lengthening of the diastolic interval between action potentials in the presence of the combination and may partially account for a lack of enhanced rate-dependent block in the presence of the combination versus quinidine alone.

Characteristics of recovery from frequency-dependent block. Recovery from frequency-dependent block after a train of action potentials was determined for each drug and their combination. After a steady reduction of Vmax had been obtained following the stimulus train, rapid stimulation was terminated and Vmax values of single action potential upstrokes elicited at various times during the ensuing rest recovery period were determined. The increasing Vmax values obtained during the recovery period were plotted against the elapsed recovery time. The time course of Vmax recovery was fitted to one or more exponential curves and the time constant(s) determined.

A recovery period of 5 sec was usually sufficient for Vmax to recover completely in the presence of tocainide. However, a recovery period of 30 sec was required in the presence of either quinidine or the combination. If there were more than a few millivolts of depolarization during the 30 sec recovery interval the results were not used.
FIGURE 4. Comparison of the effects of quinidine (5 µM) and a combination of tocainide (50 µM) on the magnitude and rate of development of frequency-dependent block. V\textsubscript{max} (top), V (middle), and the take-off potential (TOP; bottom) are plotted for each action potential during the stimulus train under control conditions (+), 35 and 60 min after exposure to quinidine (Δ), 30 min after exposure to the combination (○), and 30 min after washout of the combination with quinidine alone (▲). The V\textsubscript{max} curves represent nonlinear least square fits to the data points. The magnitude of rate-dependent I\textsubscript{T H block was 8.0% after a 35 min exposure to quinidine, 12.63% after a 1 hr exposure to quinidine, 9.32% after exposure to the combination, and 10.22% after reexposure to quinidine alone. Decline of V\textsubscript{max} in the presence of quinidine approximated a single exponential with rate constants of 7.41 and 7.0 beats after 35 and 60 min of initial exposures to drug and 5.5 beats after reexposure. Decline of V\textsubscript{max} during exposure to the combination followed a double exponential, with rate constants of 0.71 and 7.2 beats for the first and second exponential, respectively. V and TOP were essentially constant throughout the stimulus train.

FIGURE 5. Rate-dependent effects on APD at the 95% level of repolarization in the presence of quinidine versus the combination. Data were obtained from the same preparation as in figure 4. Under all conditions, the duration of the first action potential of the train was prolonged. Shortening of duration during the train followed a double exponential decline, with rate constants of 0.32 and 9.1 pulses under drug free condition (+), 1.14 and 8.71 pulses after exposure to quinidine (Δ), 0.89 and 9.34 pulses after exposure to the combination (○), and 0.95 and 7.67 pulses after reexposure to quinidine alone (▲). Every second point has been deleted for the sake of clarity.
We could not accurately determine recovery time constants under drug-free conditions because $V_{\text{max}}$ had already attained its maximal value at the time when repolarization was complete. However, when this measurement was made during the terminal phase of repolarization a time constant of $26.14 \pm 10.45$ msec (mean $\pm$ SD, $n = 7$) was obtained. This value for control recovery time constant (although it includes both voltage- and time-dependent recovery) is compa-

![Figure 6](http://circ.ahajournals.org/)

**FIGURE 6.** Time course of recovery from block in the presence of 50 $\mu$M of tocainide. Top, $V_{\text{max}}$ versus diastolic interval, where zero diastolic interval is defined as return of repolarization to the maximum diastolic potential. Bottom, Activation time; i.e., the time from the beginning of the stimulus pulse to $V_{\text{max}}$. Although the activation time is a measure of excitability and conduction, it does provide some approximation of drug effect on conduction. Recovery from block in the presence of drug was assessed when the extracellular potassium concentration was 4, 6, and 8 mM. The curves represent nonlinear least squares fits of the data. Under drug-free conditions, the recovery time constant was 27 msec. Under tocainide and a 4 mM $K^+$ concentration, the recovery time constant was 1.08 sec and the membrane potential was 87.0 mV. Under 6 and 8 mM $K^+$ concentrations, the membrane potential decreased to 83.5 and 79.5 mV, respectively, and the time constants increased to 1.28 and 2.36 sec, respectively. Early diastolic block, defined as the magnitude of $V_{\text{max}}$ depression at zero diastolic interval compared with recovery $V_{\text{max}}$, was 11.5%, 15.7%, and 20.9% at 4, 6, and 8 mM $K^+$, respectively. Early diastolic block was estimated by extrapolating the exponential fit back to zero time. The time constant of $V_{\text{max}}$ recovery was reflected in a similar time course of change in activation time, namely 0.015, 1.21, 1.33, and 2.18 sec for control and tocainide at 4, 6, and 8 mM $K^+$, respectively, suggesting that the time course of $V_{\text{max}}$ recovery parallels the time course of changes in conduction. At 4 mM $K^+$, $V_{\text{max}}$ recovered to drug-free values at long cycle lengths. However, at 6 and 8 mM $K^+$, $V_{\text{max}}$ was considerably depressed even at long cycle lengths, suggesting substantial amounts of tonic block (21.6% and 37.8% respectively).
Vmax recovery was delayed by both drugs. Unblocking following the train proceeded most rapidly with tocainide. A typical example is shown in figure 6. The time constant for recovery of Vmax averaged 1.04 ± 0.49 sec (mean ± SD, n = 8). Early diastolic block, defined as the magnitude of Vmax depression at zero diastolic interval compared with recovery Vmax, averaged 10.34 ± 3.10% (mean ± SD, n = 8).

Both the time constant for recovery of Vmax and the magnitude of early diastolic block produced by tocainide was highly dependent on membrane potential (figures 6 and 7). The less negative the membrane potential the slower was the recovery time constant and the greater the early diastolic block, suggesting that at reduced membrane potentials, a greater number of sodium channels were blocked and drug unbinding was slower. There was approximately a sixfold difference in the time constant for recovery of Vmax and approximately a fourfold difference in the magnitude of early diastolic block over approximately a 15 mV range of membrane potentials.

Figure 8 compares the recovery kinetics of Vmax in the presence of quinidine versus a combination of quinidine and tocainide. Poststimulation recovery from quinidine-induced block was characterized by a single time constant while that from block induced by the combination was characterized by two time constants. The mixture of the two drugs produced a much larger depression of Vmax of early premature responses compared with that by quinidine. There was also additional depression of Vmax (8.5%) at long coupling intervals. However, there was no additional depression of activation time at these same long intervals. The degree of depression of Vmax at long coupling intervals after exposure to the combination was variable, ranging from minimal block to about 10% block. However, the depression of early responses was always consistently greater. During recovery from drug-induced frequency-dependent block, changes in activation time were found to be reciprocally related to changes in Vmax. The time constant for recovery of Vmax in the presence of quinidine averaged 4.81 ± 0.76 (mean ± SD, n = 8). The two time constants for recovery of Vmax in the presence of the combination were 0.43 ± 0.22 and 5.94 ± 0.56 sec (mean ± SD, n = 5). The first time constant of the combination presumably corresponds to that of tocainide while the second time constant presumably corresponds to that of quinidine. The amount of block present at the beginning of diastole (early diastolic block) was significantly greater in the presence of the combination (22.57 ± 9.23%) than in the presence of quinidine alone (16.37 ± 5.72%; p < .05). The large depression of Vmax of early premature responses was consistently accompa-

![Graph](image.png)

**FIGURE 7.** Correlation between time constant of recovery ($T_{rec}$) of Vmax, % early diastolic block (%EDB), and membrane potential in the presence of 50 µM of tocainide. The graph shows the results from seven preparations. Membrane potential was varied by exposing the preparation to a Tyrode's solution in which the potassium concentration was increased to either 6 or 8 mM. Time constants of recovery were determined during superfusion with control Tyrode's solution (4 mM K+) and redetermined after exposure to high-potassium Tyrode's solution. Membrane potential ranged from −90.4 to −77 mV in the seven preparations; there was almost a sixfold difference in $T_{rec}$ of Vmax over this range of membrane potentials and approximately a fourfold difference in the magnitude of early diastolic block. Both correlations were significant at p < .05.
nied by an increase in activation time, suggesting that the depression of $V_{\text{max}}$ of early responses reflects a slowing of conduction.

In two experiments, the concentration of tocainide in the combination was increased to 100 and 200 $\mu$M. There was no change in the time constants of recovery of $V_{\text{max}}$ but a progressive increase in the amount of block present at the beginning of diastole did occur. The greater magnitude of block was due to more block by tocainide. The quinidine component of the block remained the same, suggesting that greater early diastolic block was due to the additive effects of both drugs.

The membrane potential dependence of the time constant for recovery of $V_{\text{max}}$ could not be determined in the presence of quinidine because of the long recovery intervals required and the likelihood of diastolic depolarization at these long intervals. At a normal extracellular potassium concentration and membrane potentials greater than $-85$ mV, a few millivolts of depolarization did not prevent recovery of $V_{\text{max}}$ at long diastolic intervals. However, when the preparations were depolarized by increasing the extracellular potassium concentration, even a small amount of depolarization (1 to 2 mV) prevented full $V_{\text{max}}$ recovery and thus recovery time constants could not be accurately determined.

$V$, the potential at which $V_{\text{max}}$ occurs, remained essentially constant both during onset and recovery from block. $V$ only changed if $V_{\text{max}}$ decreased as a result of membrane depolarization. Since membrane potential remained fairly constant (varying by no more than a few millivolts) during onset and recovery from block, $V$ would be expected to remain constant. Thus, reduction of $V_{\text{max}}$ cannot be attributed to changes in sodium driving force ($V_m - V_{Na}$) at the time of $V_{\text{max}}$.

**Voltage-dependent effects.** In nine experiments we assessed the effects of tocainide, quinidine, and their combination on the relationship between $V_{\text{max}}$ or activation time and membrane potential. Tocainide con-

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**FIGURE 8.** The time course of recovery of $V_{\text{max}}$ and activation time in the presence of 5 $\mu$M quinidine and 30 min after switching to a combination of 5 $\mu$M quinidine plus 50 $\mu$M tocainide. $V_{\text{max}}$ (top), activation time (second panel), $V$ (third panel), and takeoff potential (TOP; bottom) are plotted against diastolic interval. The curves represent nonlinear least squares fits of the data. Recovery in the presence of quinidine followed a single exponential with time constants of 4.96 and 5.03 sec for $V_{\text{max}}$ and activation time, respectively. Recovery in the presence of the combination followed a double exponential with time constants of 0.37 and 5.39 sec for $V_{\text{max}}$ and 0.44 and 6.20 sec for activation time. Early diastolic block, defined as the magnitude of $V_{\text{max}}$ depression at zero diastolic interval, was 9.14% in the presence of quinidine and 16.80% under the combination. Of the latter, 10.18% was attributable to quinidine, suggesting that the two drugs had an additive effect. Both $V$ and TOP remained essentially constant throughout the diastolic interval.
sistantly shifted the $V_{\text{max}}$ curves to more negative potentials, suggesting an interference with sodium inactivation. This effect developed at stimulation rates at which tocainide shows no frequency-dependent effects. Figure 9, A shows that under control conditions, half inactivation occurred at $-71$ mV. Tocainide, 50 $\mu$M, shifted the steady-state inactivation curve along the voltage axis to more hyperpolarized potentials, half inactivation now occurring at $-75$ mV. The peak $V_{\text{max}}$ obtainable was diminished slightly by tocainide suggesting that there was a small degree of resting block (less than 3%) at membrane potentials more negative than $-90$ mV. Tocainide shifted the curve an average of 3.25 mV ($n = 2$).

In contrast to tocainide, 5 $\mu$M of quinidine produced little or no shift in the $V_{\text{max}}$--membrane potential

![Graphs showing relationships between $V_{\text{max}},$ activation time, and membrane potential under control conditions and in the presence of 50 $\mu$M tocainide.](http://circ.ahajournals.org/)

**FIGURE 9.** A. Relationship between $V_{\text{max}},$ activation time, and membrane potential under control conditions (+) and in the presence of 50 $\mu$M tocainide (-). Top, Actual $V_{\text{max}}$ values. Middle and bottom, $V_{\text{max}}$ and activation time have been normalized to their respective values at the most negative membrane potential. Membrane potential was decreased by increasing the K+ concentration in the superfusate from 2.5 to 12 mM in 1 to 2 mM steps until the preparation stopped responding. Equilibration with each concentration was attained before switching to the next higher concentration. Stimulus cycle length was 10 sec. Tocainide shifted the curves 4 mV in the hyperpolarizing direction. B. Same relationship as in A under control conditions (+) and after exposure to 5 $\mu$M quinidine ($\Delta$) and a combination of 5 $\mu$M quinidine and 50 $\mu$M tocainide (○), but in a different preparation than in A. While quinidine shifted the curves minimally, the combination produced an almost 4 mV shift. This shift was similar to that produced by tocainide alone. Stimulus cycle length was 10 sec.
curve (figure 9, B). Addition of tocainide to fibers equilibrated with quinidine produced a shift similar to that produced by tocainide alone in control fibers, as seen in figure 9, A. In the presence of the combination, there was a greater depression of Vmax, particularly at more depolarized membrane potentials. The combination shifted the curve an average of 3.15 ± 0.11 mV (n = 4).

Discussion

In a beating heart, interaction of local anesthetic type drugs with the sodium channel receptor changes dynamically throughout the cardiac cycle. During an action potential, sodium channels cycle through three different states, rested, open and inactivated, as described by Hodgkin and Huxley.20 Hondeghem and Katzung1 proposed that each one of these three states can interact with antiarrhythmic drugs and that drug-associated channels also rotate between the three states but they behave as if their voltage dependence is shifted to more hyperpolarized potentials.

Most class I antiarrhythmic drugs have a very low affinity for the receptor site when the channel is in the rested state, but have a high affinity for the receptor when the channel is open or inactivated.2 Thus, block develops during each action potential and then dissipates when the channels return to the rested state at the beginning of diastole. The rate of drug dissociation during diastole varies for different clinically effective class I drugs.21 Thus, sodium-channel block evoked by these drugs is characterized by a pronounced dependence on stimulation rate. Our results suggest that for quinidine, the rate of drug dissociation at normal diastolic potentials is slow enough (4.0 to 6.1 sec) so that block does not fully dissipate between beats throughout the clinical range of heart rates. Similar time constants of dissociation for quinidine have been obtained by others.22–24 For tocainide, the rate of drug dissociation in fully polarized cells is much faster (0.5 to 1.7 sec). Thus, in the presence of tocainide, a substantial amount of block can dissipate between beats at slow heart rates. Similar time constants of dissociation for tocainide were obtained by Gintant et al.25 in Purkinje fibers, and by Courtney26 in guinea pig papillary muscles. Oshita et al.,27 using two different experimental protocols, found two components of recovery for tocainide in guinea pig papillary muscle. It is unlikely that their first component is recovery from drug effect since they conducted their study at a basic cycle length of 4000 msec, which is unlikely to produce any degree of block in the presence of tocainide. Thus, there could not have been any block from which to recover. The first component is more likely recovery from slow inactivation, as described by Clarkson et al.28 Their second component, which is longer than that measured by us, was obtained at higher extracellular potassium concentrations.

In normal fully polarized cells, tocainide was more effective in depressing early premature impulses or those elicited at rapid heart rates, but produced much less block at slow heart rates. Quinidine, on the other hand, produced less depression of early beats and a more uniform block throughout the clinical range of heart rates.

When administered in combination at clinically effective concentrations, the magnitude of the interaction between tocainide and quinidine was highly dependent on the heart rate and the membrane potential. Tocainide depresses Vmax and delays conduction at rapid heart rates and at more depolarized membrane potentials. At depolarized potentials, depression of Vmax is due to a prolongation of the recovery time constant and possibly a greater magnitude of tonic block. At membrane potentials in the range of −75 mV, the rate of tocainide dissociation slows to the rates observed with quinidine at normal diastolic potentials. Unlike tocainide, quinidine in therapeutic concentrations produces little or no shift in the curve relating Vmax to activation voltage. Both Colatsky29 and Weld et al.24 found a minimal shift of this curve in voltage-clamped Purkinje fibers at much higher concentrations of quinidine (5 to 10 μg/ml). Thus, much higher concentrations than those effective clinically are required to produce a voltage shift of inactivation. We were unable to determine time constants of recovery for quinidine at depolarized potentials in the present study. Weld et al.24 found that unbinding of quinidine from resting sodium channels is, in fact, strongly voltage dependent. However, in the −90 to −70 mV range of membrane potentials, the time constant for unbinding from resting channels for quinidine changes only from about 4 to 7 sec (their figure 9). This is in contrast to a four- to sixfold difference in the time constant for recovery of Vmax for tocainide observed over a similar range of membrane potentials in the present study. In this respect tocainide resembles lidocaine and mexiletine, another lidocaine congener, which have been shown to produce similar voltage dependence of the time constant for recovery of Vmax.27, 30, 31 Thus, at clinical concentrations the effect of quinidine is dependent mostly on heart rate while that of tocainide is dependent on both heart rate and membrane potential.

Thus, when tocainide is combined with quinidine,
the magnitude of the block produced by this combination at low heart rates in normal fully polarized cells (greater than −90 mV) is not much greater than that produced by quinidine alone. However, at high heart rates and particularly in depolarized cells, the magnitude of block produced by the combination is very much greater than that produced by quinidine alone. The greater amount of early diastolic block is due to an additive effect of both drugs. Similar interactions have been demonstrated in vitro with combinations of lidocaine and quinidine and lidocaine and propafenone.

The state dependence of drug-channel interaction can influence the interaction between two class I drugs. Steady-state block caused by a drug with a high affinity for the inactivated site will be dependent on the ratio of the APD to diastolic interval. Block caused by a drug with a high affinity for the open channel will be influenced by rate, but not by APD. Several lines of evidence support the view that quinidine binds preferentially to open channels. There are no data available on the relative affinities of tocainide for activated and inactivated channels. If this drug resembles lidocaine, it may have a high affinity for inactivated channels. If there is substantial inactivated channel block, shortening of APD by tocainide will decrease the relative degree of block produced by the combination. In addition to a prolonged diastolic interval, this may be an additional reason why we did not observe greater rate-dependent block in the presence of the combination versus quinidine alone at an interstimulus interval of 600 msec.

**Clinical implications.** The above described interactions occur at clinically relevant heart rates and at clinically relevant concentrations. The combination of tocainide and quinidine may be beneficial for several reasons. In the presence of quinidine, addition of tocainide can provide an extra depression of conduction of early extrasystoles by blocking those channels that are not occupied by quinidine while producing little further depression of basic beats since block of sodium channels by tocainide would be expected to dissipate rapidly. Such a combination may be beneficial in suppressing early extrasystoles that contribute to the generation of a reentry mechanism.

The prolongation of the time constant of recovery of Vmax at depolarized membrane potentials and a shift of the Vmax–membrane potential relationship to more hyperpolarized potentials by the tocainide component of the combination may have important implications in the treatment of ischemia-induced arrhythmias. The combination would be expected to produce a greater depression of conduction in localized ischemic depolarized tissue without causing as much further depression in normal fully polarized tissue in the rest of the heart compared with that produced by quinidine alone. Thus, unidirectional block in partially depolarized ischemic tissue, which is conducive to reentrant arrhythmias, may be converted to bidirectional block, resulting in abolition of the reentrant mechanism.

A drug such as tocainide that selectively blocks conduction in depolarized tissue and also shortens APD can also facilitate induction of reentrant arrhythmias. The advantage of combining such a drug with quinidine is that selective depression of conduction in depolarized tissue is combined with prolongation of refractory period in fully polarized tissue.

The combination may also have beneficial effects in the treatment of arrhythmias due to enhanced automaticity. A combination of tocainide and quinidine suppresses Purkinje fiber automaticity much more than quinidine alone. The escape intervals following a train of stimuli were very much longer in the presence of the combination compared with those with quinidine alone.

Tocainide may enhance the antiarrhythmic action of quinidine while at the same time preventing quinidine-induced toxicity. A quinidine-induced increase in the corrected QT interval, particularly in the presence of slow heart rates and a low potassium concentration, has been associated with torsades de pointes—type of arrhythmias. Roden and Hoffman have shown that at cycle lengths greater than 4000 msec in low extracellular potassium, quinidine produced early afterdepolarizations in canine Purkinje fibers. Early afterdepolarizations were eliminated by interventions that abbreviated the APD. They suggested that this form of triggered activity may play a role in quinidine induced torsades de pointes. We also found that tocainide, by preventing the marked prolongation of APD by quinidine, particularly at slow heart rates and low potassium concentrations, abolished early afterdepolarizations produced by quinidine (figure 10). Hence, a combination of tocainide and quinidine might be expected to prevent the development of quinidine-induced torsades de pointes arrhythmias.

Thus, the present study provides a rationale for a possible enhanced antiarrhythmic efficacy of a combination of two class I agents that have different kinetics of interaction with the sodium channel. Clinical instances often occur in which drugs with rapid kinetics are not quite effective and maximum tolerated doses of...
drugs with slow kinetics do not achieve the required suppression of extrasystoles. Thus, a combination of class I drugs having slow and rapid recovery kinetics may provide therapeutic efficacy not obtainable with either drug alone.

The beneficial effects of such a combination for suppression of ventricular extrasystoles has already been demonstrated in two clinical studies. In the study of Duff et al., quinidine was combined with another lidocaine congener, mexiletine. Although tocainide has rapid recovery kinetics compared with quinidine, its kinetics are slower than those of lidocaine or mexiletine. This is probably related to tocainide’s poor lipid solubility and thus more restricted access to the receptor in the channel.

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FIGURE 10. Abolition of early afterdepolarizations (EADs) induced by 10 μM of quinidine in a preparation superfused with a modified Tyrode’s solution (2.7 mM potassium, 12 mM NaHCO₃) and driven at a basic cycle length of 8 sec. Top, EAD’s were obtained within 5 min of switching to a low rate of stimulation and continued in a stable alternating fashion. Middle, EADs were abolished after switching to a combination of 10 μM of quinidine and 50 μM of tocainide. Abolition was accompanied by shortening of APD. Bottom, EADs were reestablished after switching back to quinidine alone. All potentials shown are digitized traces. Similar results were obtained in three other experiments.
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