Effects of Flecainide on Ectopic Atrial Automaticity and Conduction

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Background. Previous studies have shown that class Ic antiarrhythmic agents are effective in suppressing ectopic atrial rhythms and accessory pathway conduction.

Methods and Results. To explore the potential mechanisms for their effectiveness, we investigated the concentration-dependent effects of the Ic agent flecainide acetate (0.5 to 10 μg/mL) on atrial ectopic automaticity and exit conduction in isolated rabbit tricuspid valves. This experimental model consists of three major cell types as defined anatomically and by intracellular recordings: pacemaker, transitional, and working atrial muscle. Simultaneous recordings from these cell types before and during flecainide superfusion (n=7) showed that the drug produced a slight, concentration-dependent slowing of pacemaker-transitional conduction but elicited third-degree transitional-working atrial muscle block in six of seven preparations at 10 μg/mL. Flecainide caused a significant dose-dependent reduction in the initial phase of diastolic depolarization of pacemaker cells but produced only a small, biphasic change in spontaneous pacemaker cycle length. It also caused a significant prolongation in action potential duration in pacemaker and transitional cells and reduction in upstroke velocity in atrial cells. Of note in four additional preparations, flecainide caused a concentration-dependent upward shift in the strength-duration curve for atrial fibers.

Conclusions. These data suggest that flecainide has little direct effect on ectopic atrial automaticity but rather causes exit conduction slowing and block between transitional and atrial muscle fibers. The mechanism for the induction of block is likely due to a decrease in atrial excitability creating a greater electrical load on generated impulses. (Circulation. 1993;88[part 1]:1878-1884.)

Key Words • antiarrhythmia agents • flecainide

Clinical and experimental studies have shown that Na+ channel-blocking drugs, especially those classified as Ic agents,1 are effective in suppressing ectopic atrial rhythms2-4 and in slowing or blocking accessory pathway conduction.5,6 The mechanisms underlying the effectiveness of these agents in a wide spectrum of tachyarrhythmias are not entirely known, although they are thought to be related mainly to conduction slowing and to changes in refractoriness.8 For example, recent electrophysiologic studies have demonstrated that the Ic agent flecainide acetate prolongs nearly all cardiac conduction intervals with particularly marked effects on His-Purkinje and intraventricular conduction.9 Although its effect on refractoriness in normal tissue is relatively small, flecainide significantly prolongs the refractory period of accessory pathways,8 making this a particularly effective agent in the treatment of reentrant tachycardias that use such tissues.

To further explore the mechanisms of the antiarrhythmic actions of class Ic agents, the cellular electrophysiologic effects of flecainide were examined in an in vitro experimental model of ectopic atrial automaticity. The model consisted of the isolated rabbit tricuspid valve, which contains three distinct types of cellular responses and enabled a quantitative examination of exit conduction from ectopic foci.10-12 The objectives of these studies were to delineate the effects of flecainide acetate with respect to the cellular electrophysiologic characteristics of pacemaker, transitional, and working atrial fibers and to assess the exit conduction characteristics of automatic impulses. Portions of this investigation have been presented in abstract form.13

Methods

Experimental Preparation: Intracellular Recordings

New Zealand rabbits weighing 1.5 to 2.5 kg were euthanized with sodium pentobarbital (100 mg/kg IV), and their hearts were quickly removed through a mid-sternal incision and placed in room-temperature Tyrode’s solution gassed with 95% O2-5% CO2. Unless otherwise stated, the Tyrode’s solution for all experiments contained the following (in mmol/L): NaCl 129, KCl 4, NaH2PO4 0.9, NaHCO3 20, CaCl2 1.8, MgSO4 0.5, and glucose 5.5 (pH 7.4). The right ventricle was opened, after removing the apex, by an incision made through the free wall. The chordae tendinea were then cut, and nonseptal tricuspid valve leaflets were excised along with approximately 5 to 7 mm of adjacent working atrial muscle. Isolated valve preparations, measuring approximately 1.0 to 1.5 cm2, were pinned atrial side up to the floor of a 2-mL capacity tissue bath perfused with warmed (37°C), oxygenated Tyrode’s solution at a rate of 5 mL/min.
Transmembrane potentials from valve cardiac fibers were monitored (WPI model KS-700) using standard microelectrode techniques. Amplified signals were recorded on FM tape (TEAC model XR-310) and later displayed on a strip-chart recorder (Gould 2600S) for measurement of several parameters that are outlined below. In some experiments, analog signals were digitized (Keithley model 570) and measured offline by computer (Compaq 286). The location of leading pacemaker fibers in each valve preparation was determined by mapping the site of earliest activation with a microelectrode referenced to a stationary extracellular bipolar electrode placed elsewhere on the leaflet. Preparations that appeared to have more than one focus were discarded.

In the present study, the electrophysiologic properties and cell-to-cell interactions of three fiber types (Fig 1) were examined: pacemaker (P), transitional (T), and atrial muscle (A). The classification of these fiber types was based on action potential morphology and anatomic location. Specifically, leading pacemaker fibers were located toward the free margin of the valve leaflet and exhibited marked diastolic depolarization with a characteristic biphasic time course.\(^ {10,11} \) Action potentials typical of working atrial fibers were recorded several millimeters from the leaflet and were characterized by a rapid upstroke (>150 V/s) initiated from potentials negative to \(-75 \) mV. Moreover, those transitional cardiac fibers interposed between pacemaker and atrial muscle fibers exhibited an intermediate action potential morphology characterized by a prominent afterhyperpolarization but an otherwise steady diastolic potential ranging from \(-60 \) to \(-70 \) mV.

Ectopic pacemaker preparations exhibited stable spontaneous activity, although in many cases exit conduction from identified pacemaker foci was blocked unless \(\beta \)-adrenergic agonists are added to the superfusate.\(^ {10,12,14} \) In the present study, exit block was evident in 8 of 13 valves (61%) superfused with normal Tyrode’s solution. Thus, to effectively analyze the influence of flecainide on conduction, those valves with documented exit block after equilibration were superfused with increasing concentrations of norepinephrine until a basal value was found supporting 1:1 exit conduction. Once determined, this concentration was kept constant throughout the ensuing experiment. Stable 1:1 exit conduction was achieved with background norepinephrine concentrations of from 0.005 to 0.5 \(\mu \)g/mL (mean, 0.11 ± 0.04 \(\mu \)g/mL).

For most experiments, simultaneous impalements were made in pacemaker, transitional, and atrial fibers in an approximate linear orientation. Once control data were obtained, the recording sites were not changed during subsequent exposures to flecainide that was superfused at cumulative concentrations from 0.5 to 10 \(\mu \)g/mL. When reimpalement was necessary at a given site, it was made at approximately the same location. The total exit conduction time from ectopic foci was subdivided into P-T and T-A components by measuring the time intervals between the midpoints of the upstrokes of simultaneously recorded action potentials. In some experiments, distances between recording sites were measured by ocular micrometer to calculate local conduction velocities. Moreover, the direct effects of flecainide on valve cells were analyzed by measuring several parameters from recorded potentials. These included maximum diastolic potential (MDP), resting membrane potential (RMP; for atrial fibers), maximum rate of rise of the upstroke (\(V_{\text{max}}\); determined electronically), action potential overshoot and duration measured at 50% repolarization (APD\(_{50}\)), and spontaneous pacemaker cycle length (PCL). The latter, which was measured directly from pacemaker fiber recordings, was characterized by two distinct phases of diastolic depolarization: a brief primary phase of relatively steep slope and a secondary phase of lesser slope.\(^ {10,11} \) The slopes of these two phases, represented by \(D_1 \) and \(D_2 \), respectively, were measured graphically by tangents drawn through each (see Fig 3A).

In four separate experiments, flecainide-induced changes in atrial excitability were assessed by measuring the minimal current required to elicit a propagated response as a function of pulse duration. In these studies, valve preparations were stimulated at a cycle length of 750 milliseconds by basic pulses applied through a bipolar electrode placed in the atrial region. During basic drive, current threshold (\(I_{\text{th}} \)) was measured late in diastole by a test pulse delivered after every 15th basic stimulus. The duration of the test pulse was systematically varied, and the amount of current required to initiate action potentials was recorded to generate strength-durations curves. \(I_{\text{th}} \) measurements were normalized relative to the amount of current required to initiate action potentials under control conditions with a standard long duration test pulse (6 milliseconds).

**Statistical Analysis**

Results are expressed as mean ± SEM. Statistical comparisons of two groups were made using a Student’s \(t \) test or nonparametric rank-order test.\(^ {15} \) Simultaneous comparison of more than two groups was carried out by analysis of variance. When a significant difference among groups was indicated by the initial analysis, individual paired comparisons were made using a mod-
ified t test. Differences were considered significant at \( P < .05 \).

**Results**

**Flecainide Effects on Exit Conduction**

Under control conditions, exit conduction from automatic foci in the valve model was generally nonuniform as evidenced by markedly different P-T and T-A conduction times. This is illustrated in Fig 1, which shows simultaneously recorded action potentials from pacemaker (top trace), transitional (middle trace), and atrial sites. In this example, P-T conduction time (54 milliseconds) was more than five times greater than that for T-A (10 milliseconds), even though the distances between P-T and T-A recording sites were nearly identical (=1.5 mm). Consequently, P-T conduction velocity (0.028 m/s) was considerably less than for T-A (0.15 m/s). When measured in eight valve preparations in the absence of flecainide, local P-T conduction velocity was significantly less than that for T-A (0.036±0.008 versus 0.121±0.033 m/s, respectively; \( P < .05 \)). This illustrates the fact that exit conduction from automatic ectopic foci is usually discontinuous and slow, not only in valve preparations but also in other models of ectopic automaticity. It should be noted that conduction velocities in those preparations with background norepinephrine (n=5) did not differ statistically from those without norepinephrine (n=3) for P-T (0.026±0.004 versus 0.054±0.016 m/s, respectively) or T-A (0.112±0.04 versus 0.134±0.071 m/s, respectively) conduction.

Despite the discontinuous, slow nature of P-T conduction, flecainide had a relatively greater influence on T-A conduction. This is shown in the example of Fig 2, which displays recorded potentials using the same format as Fig 1. Fig 2A shows control traces in a preparation where P-T and T-A recording sites were separated by 1.6 and 2.3 mm, respectively. With the cumulative addition of flecainide to the superfusate at concentrations up to 2 \( \mu \)g/mL, T-A conduction time gradually increased, whereas after 5 minutes of 5 \( \mu \)g/mL, as shown in Fig 2B, third-degree block developed between transitional and atrial sites. Note that in this example, spontaneous pacemaker activity continued in the presence of flecainide but that the transitional action potential was reduced in amplitude compared with control. On washout of the drug (Fig 2C), T-A conduction was restored, although it remained markedly delayed such that electrotonic humps were evident at the transitional site.

The concentration-dependent effects of flecainide on exit conduction from ectopic foci were examined in a total of seven preparations, of which three were studied in the absence of background norepinephrine. The results of all seven experiments are shown in Table 1, which compares P-T and T-A conduction times, expressed as percent of control, at all flecainide concentrations. The occurrence of third-degree exit block for a specific flecainide concentration is also indicated. Note that flecainide impaired T-A conduction to a greater extent than for P-T conduction, with several preparations exhibiting third-degree exit block. At 10 \( \mu \)g/mL, flecainide elicited P-T block in two experiments, whereas six of seven preparations exhibited T-A block at this concentration. Although not studied systematically, 1:1 entrance conduction was present in valves during third-degree exit block, the latter of which was reversed on washout of the drug.

**Cellular Electrophysiologic Effects of Flecainide**

Flecainide induced marked changes in action potential morphology of pacemaker fibers yet did not significantly alter inherent automaticity. This is shown in Fig 3, which summarizes the concentration-dependent effects of flecainide on diastolic depolarization in pacemaker cells (n=7). Fig 3A illustrates a typical pacemaker action potential, showing the method of measuring the parameters summarized below. Fig 3B plots the slopes of the two characteristic phases of
diastolic depolarization in these fibers, designated D1 (filled circles) and D2 (open circles), respectively. As Fig 3B illustrates, a cumulative increase in flecainide concentration was associated with a significant decline in the slope of phase D1 compared with control yet little change in phase D2. Moreover, PCL (Fig 3C) varied in a biphasic manner as a function of flecainide concentration, increasing from control up to 1 µg/mL flecainide, then shortening at 2 µg/mL, and again lengthening at higher concentrations. Despite these changes, however, PCL was not statistically different at any of the flecainide concentrations tested when compared with control.

Table 2 summarizes the electrophysiologic effects of flecainide on all three major cell types in ectopic foci studied in the present model. Note that flecainide had little direct effects on pacemaker and transitional fibers, other than a concentration-related increase in APD50. In contrast, flecainide significantly reduced Vmax and overshoot in atrial fibers but did not change APD50 (note that data for atrial fibers were obtained under paced conditions). These changes in atrial fibers elicited by flecainide are consistent with its Na+ channel-blocking effects and suggest that a decrease in atrial excitability may have been the primary determinant of exit block. To examine this possibility, strength-duration curves were analyzed in four additional valve preparations as a function of flecainide concentrations. For each preparation in this series, bipolar stimuli were applied to working atrial muscle. The results of these experiments are shown in Fig 4, which plots mean normalized current intensity (IA/Vmax) on the ordinate as a function of test pulse duration. Compared with control (filled circles), flecainide shifted the strength-duration curve upward by an amount proportional to its concentration. Although not shown in this figure, 5 and 10 µg/mL flecainide produced even greater shifts in the curve above that shown for 2 µg/mL (open squares).

**Discussion**

**Flecainide as a Class Ic Agent**

Clinical and experimental investigations have shown that the effectiveness of flecainide in suppressing a wide spectrum of tachyarrhythmias is related mainly to conduction slowing and changes in refractoriness. Specifically, recent electrophysiologic studies have demonstrated that the drug prolongs nearly all cardiac conduction intervals with particularly marked effects on His-Purkinje and intraventricular conduction. On the basis of these actions, therefore, flecainide has been categorized as a class Ic antiarrhythmic drug. However, although it appears to have a slight effect on refractoriness in normal tissue, flecainide significantly prolongs the refractory period of accessory pathways, making this a particularly effective agent in the treatment of reentrant tachycardias that use such tissues.

The mechanisms underlying the slowing of conduction by flecainide have recently been examined in detail at the cellular level where it has been shown that the Na+ channel is a major target for its effects. In isolated
include the modulation of other cellular electrical properties besides Na⁺ channel activity. For example, flecainide has been shown to inhibit the calcium current (I_{Ca}) in frog ventricular myocytes. In contrast to the effects on the Na⁺ channel, flecainide in these cells blocked I_{Ca} in an apparent tonic fashion with little use dependence. In addition, flecainide has been shown to block the delayed rectifier (I_{K}) in cat ventricular myocytes, while indirect evidence in human atrial fibers suggests that it may also inhibit the transient outward current (I_{to}). These latter effects on K⁺ currents may help explain the efficacy of flecainide in the suppression of atrial fibrillation, flutter, and reentrant supraventricular tachycardias using accessory pathways.

**Flecainide-Induced Exit Block From Ectopic Foci**

Histologic and electrophysiologic correlations in ectopic atrial tachycardia in humans are limited. Wyndham and colleagues demonstrated triggered automaticity in surgically excised atrial preparations where limited mapping of the area around the focus suggested centrifugal propagation out of this region. Histologic analysis of these tissues revealed an increased connective tissue and monocyte infiltration, but no correlation was made between these histologic findings and the exit conduction characteristics of the focus. Josephson et al. were able to identify an automatic focus in surgically mapped atria and resected the site for in vitro analysis with microelectrodes. They found evidence of delayed, asynchronous propagation from the focus that was associated histologically with degenerative myocytes and mesenchymal cells. Larger studies have found that automatic foci can exist in areas with normal histology, healing myocarditis, or other anisotropic substrates where thin strands or muscle bundles are separated by fat or connective tissue. Thus, the rabbit atrioventricular valve preparation with its area of slow conduction across an anisotropic transitional zone of cardiac myocytes appears to model some of what is known about ectopic atrial rhythms in humans.

In the present investigation, flecainide was effective in suppressing manifest ectopic automaticity not by a direct inhibition of pacemaker activity but rather by eliciting exit block from ectopic foci (Fig 2 and Table 1). Moreover, exit conduction was most influenced at the junction between transitional and atrial zones compared with conduction from pacemaker to transitional regions (Table 1). This disparity of effects on the two components of exit conduction identified in this model is somewhat surprising since under control conditions conduction from pacemaker to transitional regions generally was slower than that from transitional to atrial. On the other hand, it is to be expected that flecainide would exert relatively minor direct effects on ectopic impulse generation since it has been shown in this model and in isolated valve myocytes that pacemaker fibers generate impulses by mechanisms that are TTX insensitive but suppressed by slow channel blockers. Although we did not measure I_{Ca} directly in pacemaker fibers, the lack of effect of flecainide on V_{max} (see Table 2) suggests that Ca²⁺ channels in these cells were not significantly blocked.

The mechanism of exit block produced by flecainide in this model can best be explained by its interaction with Na⁺ channels in atrial fibers resulting in the...
altered the amplitude-excitability (source-sink) relationship between slow-response fibers within the ectopic focus and surrounding working atrial myocardium. That is, block may be hypothesized to result from a decreased magnitude of excitatory current generated by cells, a decrease in excitability of fibers within the conduction pathway, or by both. In the present study, flecainide produced little change in the amplitude or Vmax of pacemaker or transitional fibers (however, see Fig 2), yet potent elicited T-A block, suggesting that its effects on excitability of atrial fibers were primarily responsible for the induction of block. Indeed, in paced preparations, flecainide produced an upward shift in measured strength-duration curves for atrial fibers, indicating that excitability was reduced (Fig 4). This situation is analogous to the findings of Joyner et al, who hypothesized that agents that reduce tissue excitability impose a greater electrical "load" on locally excited cells. In the present report, it thus is hypothesized that flecainide, predominantly by its effects on atrial fibers, increased the electrical "load" on transitional fibers to such an extent that block ensued at the junction of these two regions.

Flecainide-induced changes in the amplitude-excitability relationship may also explain its efficacy in suppressing accessory pathway conduction. In this case, it may be hypothesized that a reduction in excitability of a large tissue mass connected to a smaller accessory pathway increases the "load" on impulses conducting through the latter. Moreover, under conditions in which regenerative inward Na+ current is also reduced, it would be expected that the current generated by accessory pathway fibers would be a less efficacious stimulus for the activation of resting fibers, resulting in conduction failure.

**Direct Effects on Valve Cells**

Although flecainide significantly impaired exit conduction, it had little direct influence on the rate of intrinsic spontaneous activity of pacemaker fibers (Fig 3C). Nevertheless, the action potential morphology of pacemaker fibers was markedly altered during flecainide superfusion, particularly with regard to the initial phase of diastolic depolarization (D1; Fig 3B). This change may represent flecainide's influences on K+ conductances since it has been shown in isolated valve myocytes that the repolarization and subsequent diastolic depolarization phases are governed, at least in part, by the time-dependent K+ current, Iw. However, it remains to be determined whether this or possibly other K+ currents (eg, Is) are involved in the diastolic depolarization of ectopic pacemaker fibers. Nevertheless, our data suggest that the ionic mechanism underlying phase D1 is probably not essential in determining

TABLE 2. Cellular Electrophysiologic Effects of Flecainide on Valve Cardiac Fibers

<table>
<thead>
<tr>
<th></th>
<th>Flecainide Concentration, (\mu\text{g/mL})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>P fibers</strong></td>
<td></td>
</tr>
<tr>
<td>MDP, mV</td>
<td>69.3±2.9</td>
</tr>
<tr>
<td>Vmax, V/s</td>
<td>13.1±1.9</td>
</tr>
<tr>
<td>OS, mV</td>
<td>18.7±2.9</td>
</tr>
<tr>
<td>APD90, ms</td>
<td>46.3±2.0</td>
</tr>
<tr>
<td><strong>T fibers</strong></td>
<td></td>
</tr>
<tr>
<td>MDP, mV</td>
<td>78.6±6.9</td>
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<td>OS, mV</td>
<td>11.1±2.2</td>
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<tr>
<td>APD90, ms</td>
<td>43.3±1.0</td>
</tr>
<tr>
<td><strong>A fibers</strong></td>
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<tr>
<td>RMP, mV</td>
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<tr>
<td>Vmax, V/s</td>
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</tr>
<tr>
<td>OS, mV</td>
<td>15.7±2.2</td>
</tr>
<tr>
<td>APD90, ms</td>
<td>28.3±4.4</td>
</tr>
</tbody>
</table>

P indicates pacemaker; T, transitional; A, working atrial muscle; MDP, maximum diastolic potential; RMP, resting membrane potential; Vmax, maximum rate of rise; OS, action potential overshoot; and APD90, action potential duration measured at 50% repolarization. *P<.05, †P<.01 compared with control.

![Figure 4](http://circ.ahajournals.org/)

**Fig 4. Strength-duration relationships in atrial fibers as a function of flecainide concentration.** Normalized current intensity (Ia/Ia-control) is plotted as a function of test pulse duration. Mean data from four preparations are shown. Standard error bars have been omitted for purposes of clarity.
spontaneous activity in these cells since a significant inhibition of this phase by flecainide was not associated with a parallel change in PCL.

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