Frequency-Dependent Effects of Diltiazem on the Atrioventricular Node During Experimental Atrial Fibrillation

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Calcium channel blockers depress atrioventricular (AV) nodal properties in vivo in a frequency-dependent manner, suggesting that selective drug action during supraventricular arrhythmias may result from use-dependent properties. The present study was designed to examine whether or not the rate-dependent actions of diltiazem account for its therapeutic effects during atrial fibrillation. The determinants of the ventricular response to atrial fibrillation (concealed AV nodal conduction and AV node functional refractory period, AVFRP) were evaluated at multiple cycle lengths (with extrastimulus techniques) and during electrically induced atrial fibrillation (with indirect indexes from RR interval histograms) in anesthetized dogs. In the presence of diltiazem, AVFRP increased progressively relative to control as rate accelerated. At cycle lengths comparable to sinus rhythm in humans, AVFRP increased 10%, 17%, and 32% after doses 1, 2, and 3 of diltiazem, respectively. Drug-induced increases in AVFRP were greater at basic cycle lengths just above the Wenckebach point (17%, 48%, and 81%) and were maximal during atrial fibrillation (39%, 86%, and 154% increases for doses 1, 2, and 3, respectively). Diltiazem also increased the AV conduction system effective refractory period in a frequency-dependent manner without affecting the atrial effective refractory period, thereby increasing the potential zone of concealment into the AV node. Frequency-dependent increases in the zone of concealment were produced by diltiazem and were associated with marked increases in the standard deviation of RR interval histograms during atrial fibrillation (257%, 526%, and 923% increases after doses 1, 2, and 3, respectively). The combination of rate-dependent increases in AVFRP and zone of concealment resulted in a marked amplification of diltiazem’s effects during atrial fibrillation, with mean RR interval increases (88%, 200%, and 300% after doses 1, 2, and 3, respectively) that were 8–10 fold greater than increases in AVFRP at cycle lengths comparable to sinus rhythm in humans. We conclude that diltiazem’s frequency-dependent effects lead to highly selective depression of AV nodal function during atrial fibrillation. (Circulation 1989;80:380–389)

Electrophysiologic and negative inotropic effects of calcium channel blockers are dependent on underlying heart rate.\(^1\)–\(^3\) Prior in vitro studies have shown that maximal depression of slow inward current occurs at faster driving frequencies and with activations ending shorter diastolic intervals.\(^4\)–\(^8\) We have recently shown that verapamil, diltiazem, and nifedipine slow atrioventricular (AV) nodal conduction and prolong AV nodal refractoriness in vivo in a frequency-dependent manner and that the time course of recovery of AV conduction slowing is specific to the drug studied.\(^9\) On the basis of these observations, we hypothesized that these agents would have more profound effects on AV nodal properties during supraventricular tachyarrhythmias than during sinus rhythm. This would lead to desirable selectivity in their action during the very arrhythmias for which they are used. However, this hypothesis has not been directly tested in either spontaneous or experimentally induced arrhythmias.

Atrial fibrillation is an example of a supraventricular arrhythmia for which use-dependent drug effects
may be particularly important in determining efficacy, because atrial impulses at rates of 400–600/min result in a high input rate to the AV node. Verapamil and diltiazem have been effective in controlling the ventricular rate during this arrhythmia. The determinants of the ventricular response during atrial fibrillation include the functional refractory period of the AV node (AVFRP) and concealed AV nodal conduction resulting from intranodal impulse block. Preferential salutary effects of calcium channel blockers during atrial fibrillation could be produced by rate-dependent changes in either AVFRP or the degree of concealed conduction in the AV node.

The purpose of this study was to examine the effects of diltiazem on the ventricular response during experimental atrial fibrillation and to relate these effects to rate-related changes in functional refractoriness and concealed AV nodal conduction to determine the clinical relevance of frequency-dependent drug actions.

Methods

General

Mongrel dogs of either sex were anesthetized with morphine (2 mg/kg s.c.) and α-chloralose (100 mg/kg i.v.). Catheters were inserted into both femoral veins and arteries and were kept patent with heparinized saline (0.9%). Dogs were ventilated through an endotracheal tube with a Harvard animal respirator (South Natick, Massachusetts). Tidal volume and respiratory rate were adjusted after measurement of arterial blood gases to ensure adequate oxygenation (SaO₂ ≥ 90%) and physiologic pH (7.35 to 7.45). A thoracotomy was performed through the fourth right intercostal space. After suspension of the heart in a pericardial cradle, two bipolar Teflon-coated stainless steel electrodes were inserted into the right atrial appendage for recording and stimulation. Body temperature was monitored continuously with a thermistor within the chest cavity and was maintained at 37–38°C by a homeothermic heating blanket. A Statham P23 ID transducer (Cleveland, Ohio), electrophysiologic amplifiers, and a paper recorder (Siemens Mingograf 80, Sweden) were used to record blood pressure, electrocardiographic leads II and aVR, a right atrial electrogram, and stimulus artifacts. Stimulation was applied with 4-msec square-wave impulses at twice the diastolic threshold. The sinus node was crushed to allow for a wide range of pacing rates.

All dogs were autonomic ally blocked to measure direct drug effects without contamination by autonomic reflex changes. Vagal effects were prevented by surgical division of the cervical vagi followed by intravenous administration of 1 mg atropine. β-Blockade was produced by administration of 0.5 mg/kg atenolol. Repeated doses of atropine (0.5 mg) and atenolol (0.25 mg/kg) were administered hourly. This regimen has previously produced sustained autonomic blockade.

Experimental Protocol

Experiments were conducted to assess 1) the frequency-dependent effects of diltiazem on AV nodal refractoriness during atrial pacing and induced atrial fibrillation (eight dogs) and 2) the frequency-dependent effects of diltiazem on concealed AV nodal conduction (four additional dogs).

Atrioventricular nodal refractoriness (atrial pacing and atrial fibrillation). Wenckebach cycle length was determined under control conditions by decreasing atrial pacing cycle length by 10 msec decrements until second-degree AV block occurred. This was repeated before and after each experimental protocol to ensure stability of AV nodal function during electrophysiologic study under control conditions and during each drug infusion. The functional refractory period of the AV conduction system (AVFRP) was determined by introducing single premature stimuli (S₂) after 20 basic (S₁) stimuli. The resulting V₁V₂ interval was measured, and a curve relating V₁V₂ to the S₁S₂ interval was established. The AVFRP was defined as the shortest V₁V₂ resulting from premature atrial stimulation. This process was repeated at multiple basic cycle lengths (S₁S₂) ranging from 300 to 1,000 msec. The effective refractory period of the AV conduction system (AERP) was defined as the longest A₁A₂ failing to result in a propagated ventricular response and was determined at the same cycle lengths. The atrial effective refractory period (AERP) was also determined at a pacing cycle length of 600 msec.

After determination of the AERP, AVERP, and AVFRP, atrial fibrillation was induced by continuous atrial stimulation at 10–50 Hz. In each experiment, atrial pacing cycle length was adjusted until the resultant ventricular response was consistently irregular. PACING-induced atrial fibrillation was confirmed in each case by observing irregular atrial activity in the electrocardiographic and intracardiac recordings and by the persistence of spontaneous atrial fibrillation lasting between several seconds and several minutes after the interruption of pacing. Two minutes after induction of atrial fibrillation, a continuous electrocardiographic recording lasting 5–10 minutes was obtained. Because AV nodal conduction slowing in the presence of diltiazem requires time to reach steady state during pacing at any given rate, all determinations of refractoriness during steady-state pacing or atrial fibrillation were preceded by pacing for 2 minutes.

After control measurements were completed, incremental doses of diltiazem were infused intravenously, and the experimental protocol was repeated. The dosing regimens used were developed in previously published experiments and were designed to result in steady-state concentrations spanning the range of concentrations observed after therapeutic doses of diltiazem in humans. The elec-
trophysiologic study was repeated 10 minutes after completion of each loading dose. Before and after each experimental protocol, blood samples were obtained during the maintenance drug infusion, for subsequent measurement of plasma diltiazem concentrations. The loading and maintenance doses used and the resulting plasma concentrations are listed in Table 1.

**Concealed atrioventricular nodal conduction.** Concealed conduction was assessed in four animals by modifications of previously described methods.23,24 The right atrial appendage was paced at a constant basic cycle length. Single premature atrial stimuli (S1) were introduced after 20 basic stimuli (S1–S1). The resulting S1–V2 interval was measured and used as an index of AV nodal conduction time. This was plotted against S1–S2 and is referred to as the AV node recovery curve. The atrial and AV conduction system effective refractory periods were also determined at the same basic cycle length during the determination of the AV node recovery curve. This was performed under control conditions and after diltiazem administration. The protocol was then repeated after interpolating a nonconducted atrial impulse (S′) between the last beat of the basic drive (S1) and the test stimulus (S2). Coupling intervals were chosen so that S′ resulted in atrial activation but was blocked in the AV node (i.e., S1–S′ exceeded the atrial effective refractory period but was less than the AVERP). AVERP was determined throughout a range of S1–S′ intervals, with S1–S′ increased by 10-msec increments (from 30 msec beyond AVERP to 10 msec below the AVERP of S1). In this way, the zone between the AERP and AVERP was scanned with the interpolated atrial stimulus during repeated determinations of AVERP. An S′ that fails to penetrate into the AV node should have no measurable effect on the node, whereas an S′ that penetrates nodal tissue without exiting (i.e., is concealed) will make it more difficult for a subsequent S2 to propagate, thereby increasing AVERP.23 A 10-msec or greater increase in the AVERP of S1 after introduction of the interpolated nonconducted atrial impulse (S′) was taken to indicate concealed conduction of S′ into the AV node. The zone of concealment was defined as the range of S1–S′ intervals causing concealment.22,23 Zone of concealment was measured at basic cycle lengths ranging from 500 to 1,000 msec. During the control state, AVERP uncommonly exceeded AERP by more than 30 msec, indicating lack of a potential zone of concealed AV conduction by the definition established. Because of the complexity and potential duration of the protocol to evaluate AV nodal concealment, all measurements were performed after infusion of a single dose (dose 2) of diltiazem (Table 1).

**Data Analysis**

Electrophysiologic recordings during refractory period determinations were made at 250 mm/sec, and continuous recordings during atrial fibrillation were made at 50 mm/sec. All measurements were made with a digitizing tablet coupled to an IBM compatible microcomputer by commercially available software (Sigmascan, Jandel Scientific, Corte Madera, California). During determination of AVERP under control conditions, refractoriness of atrial tissue was sometimes limiting,25 and thus, the value obtained for AVERP was an upper limit only. After infusion of diltiazem, AVERP always exceeded AERP.

Consecutive RR intervals during induced atrial fibrillation were measured, and RR interval histograms were constructed.19 A minimum of 500 ventricular complexes were analyzed during each period of atrial fibrillation. The mean, minimum, and standard deviation of RR intervals were calculated for each histogram. The minimum RR interval during atrial fibrillation was used as an index of the func-

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**TABLE 1. Diltiazem Doses, Resulting Plasma Concentrations, and Electrophysiologic Effects**

<table>
<thead>
<tr>
<th>Dose 1</th>
<th>Dose Maintenance (µg/kg)</th>
<th>Plasma concentration (ng/ml) Before</th>
<th>Wenckebach CL (msec) Before</th>
<th>AVERP (msec)</th>
<th>AERP* (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Drug</td>
<td>200</td>
<td>3.0</td>
<td>30 ± 16</td>
<td>224 ± 33</td>
<td>189 ± 16</td>
</tr>
<tr>
<td>Dose 2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Drug</td>
<td>400</td>
<td>7.0</td>
<td>63 ± 8</td>
<td>211 ± 27</td>
<td>187 ± 27</td>
</tr>
<tr>
<td>Dose 3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Drug</td>
<td>800</td>
<td>15.0</td>
<td>210 ± 51</td>
<td>200 ± 31</td>
<td>194 ± 23</td>
</tr>
</tbody>
</table>

Results are shown for five experiments after dose 1, eight after dose 2, and five after dose 3. The loading dose was given over 10 minutes, after which the maintenance dose was begun. Electrophysiologic study was begun 10 minutes after the end of the loading dose. Results of plasma concentration determination and Wenckebach cycle length before and after electrophysiologic protocol are shown. AVERP, AERP, effective refractory periods of the atrioventricular conduction system and atrium, respectively (measured at a cycle length of 600 msec).

*p<0.05, †p<0.01 vs. control.
tional refractory period of the AV node during atrial fibrillation.10,19,26

Group data are presented as the mean±SD. Multiple comparisons between control and experimental group means were made by two-way analysis of variance with Scheffé’s test or by the unpaired t test with Bonferroni’s correction.27 Two-tailed tests were used for all statistical comparisons, and a probability of 5% or less was considered as significant.

The range of cycle lengths that could be studied was limited by the Wenckebach cycle length and spontaneous automaticity, which determined the shortest and longest pacing cycle length, respectively, under any experimental condition. Because the range of cycle lengths studied varied between experiments, we analyzed results in terms of the longest and shortest cycle length in each experiment after each drug infusion. Results in the presence of diltiazem were compared with results under control conditions at the same cycle length within each experiment. Effects of a given infusion at the longest cycle in each experiment were then grouped for statistical analysis as were effects at the shortest cycle length.

Plasma diltiazem concentration was measured by reverse-phase high-performance liquid chromatography (HPLC). Plasma samples (0.5 ml) were extracted with 0.1 ml 1N hydrochloric acid, into 2.5 ml dichloromethane to which 15 ml internal standard solution (16 µg/ml L-8040, kindly supplied by Ayerst Laboratories, Montreal, Canada) had been added. After it had been thoroughly mixed, the solution was dried under nitrogen gas and reconstituted with 45 µl mobile phase (95% methanol in water, with 0.3 ml/l glacial acetic acid and 2 g/l octanesulfonic acid). The resulting solution was injected onto a 5-µ ODS column (Chromatography Sciences, Montreal, Canada). Diltiazem was detected by a Waters ultraviolet absorbance meter at a wave length of 237 nm. The retention times for diltiazem and internal standard were 6 and 7.5 minutes, respectively, at a flow rate of 2.5 ml/min. All samples were assayed in duplicate, and a three-point standard curve was obtained in control plasma for each assay run.

Results

Plasma Concentrations of Diltiazem and Resulting Electrophysiologic Effects

Administration of incremental doses of diltiazem resulted in stable plasma concentrations ranging from 28±14 to 210±51 ng/ml (Table 1). Wenckebach cycle length and AERP were prolonged in a concentration-dependent manner after drug infusion (Table 1). No changes in AERP were observed. Drug effects, as reflected by Wenckebach cycle length, were stable during each experimental protocol, with less than 10% variation during each drug infusion.

Effects of Diltiazem on the Ventricular Response During Experimental Atrial Fibrillation

Rapid atrial stimulation induced atrial fibrillation that was continuous during stimulation and lasted for a variable length of time after cessation of pacing in all experiments. Atrial fibrillation persisted spontaneously for more than 5 minutes after pacing in three experiments. The characteristics of the ventricular response to atrial fibrillation were similar in these studies during continuous electrical stimulation compared with values during subsequent spontaneous fibrillation as indicated by mean RR interval (211±31 during stimulation and 221±38 msec after), minimum RR interval (132±27 and 147±38 msec), and standard deviation of RR intervals (40±8 and 48±40 msec). A representative example of RR interval histograms of atrial fibrillation during and after continuous pacing is shown in Figure 1.

After the infusion of diltiazem, the RR interval histogram recorded during atrial fibrillation was shifted to the right with an increase in the minimum RR interval (index for AVFRP during atrial fibrillation) and the mean ventricular response (mean RR interval) (Figure 2). The mean ventricular response was substantially slowed by the drug (Table 2). The shape of the RR interval histogram was also altered by the administration of diltiazem. In all cases, the range of RR intervals increased markedly so that the mean RR interval was prolonged to a greater extent than the minimum RR interval recorded (Figure 2). This “spaying out” of the RR interval histogram corresponded to a concentration-dependent increase in the standard deviation of histograms after drug administration (Table 2).

Effects of Diltiazem on Atrioventricular Functional Refractory Period

Figure 3 illustrates a representative experiment in which the relation between AVFRP and atrial rate
was examined before and after drug infusion. The minimum RR interval during pacing-induced atrial fibrillation under control and drug conditions is also shown. Under control conditions, AVFRP decreased consistently at shorter cycle lengths, and the shortest AVFRP (minimum RR interval) was observed during atrial fibrillation in all experiments. After the infusion of diltiazem, AVFRP was increased at all cycle lengths. In contrast to control observations, AVFRP did not decrease (e.g., dose 1, Figure 3) or increased (dose 2, Figure 3) as pacing cycle length decreased. Drug responses were evaluated at long cycle lengths (mean rates of 60–70 beats/min) and at short cycle lengths (mean rates of 111–160 beats/min) for all experiments. Table 2 summarizes the changes in AVFRP observed during atrial pacing at

**Table 2. Effects of Diltiazem on Experimental Atrial Fibrillation and Atrioventricular Refractory Period During Atrial Pacing**

<table>
<thead>
<tr>
<th>Dose 1</th>
<th>Control</th>
<th>Drug</th>
<th>Dose 2</th>
<th>Control</th>
<th>Drug</th>
<th>Dose 3</th>
<th>Control</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean RR</td>
<td>293±58</td>
<td>558±232</td>
<td>293±62</td>
<td>882±447</td>
<td>275±72</td>
<td><strong>1,058±207</strong></td>
<td>275±72</td>
<td><strong>1,058±207</strong></td>
</tr>
<tr>
<td>SD</td>
<td>46±19</td>
<td>178±143</td>
<td>51±19</td>
<td>302±212</td>
<td>47±22</td>
<td><strong>425±56</strong></td>
<td><strong>425±56</strong></td>
<td><strong>425±56</strong></td>
</tr>
<tr>
<td>AVFRP (msec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF (Min RR)</td>
<td>216±47</td>
<td>298±81</td>
<td>208±45</td>
<td><strong>387±123</strong></td>
<td>187±50</td>
<td><strong>461±75</strong></td>
<td><strong>461±75</strong></td>
<td><strong>461±75</strong></td>
</tr>
<tr>
<td>S-BCL</td>
<td>265±39</td>
<td>313±65</td>
<td>274±33</td>
<td><strong>408±69</strong></td>
<td>270±31</td>
<td><strong>491±94</strong></td>
<td><strong>491±94</strong></td>
<td><strong>491±94</strong></td>
</tr>
<tr>
<td>L-BCL</td>
<td>333±66</td>
<td>368±83</td>
<td>323±47</td>
<td>378±72</td>
<td>313±18</td>
<td>414±72</td>
<td>313±18</td>
<td>414±72</td>
</tr>
</tbody>
</table>

Results shown are for four experiments after dose 1, eight experiments after dose 2, and five experiments after dose 3. Matching control data (obtained at identical cycle lengths in the case of AVFRP at S-BCL and L-BCL) are displayed with posttreatment values. Percent change over control are shown in parentheses. Cycle lengths at which AVFRP at S-BCL was obtained averaged 375±50, 463±52, and 540±114 msec, for doses 1, 2, and 3, respectively. Corresponding cycle lengths at which AVFRP at L-BCL was obtained averaged 825±126, 863±160, and 1,000±0 msec. Mean±SD of raw data is expressed in msec.

Mean RR, mean RR interval during electrically induced atrial fibrillation; SD, standard deviation of RR intervals from the mean value during atrial fibrillation; AVFRP, functional refractory period of the atrioventricular conduction system, measured indirectly during atrial fibrillation (AF) as the minimum RR interval (Min RR) observed, during pacing at the shortest basic cycle length obtainable (S-BCL) and during pacing at the longest basic cycle length possible (L-BCL).

*p<0.05, **p<0.01, ***p<0.001 vs. control values.
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Results

(mean)

shortest

of

3

refractory period

(mean RR, solid bars) for doses 1, 2, and 3 of diltiazem. Changes in AVFRP were measured directly at the longest basic cycle length available (L-BCL), shortest basic cycle length available (S-BCL), and indirectly (as minimum RR interval) during atrial fibrillation (AF). Matched control cycle lengths were used to calculate the percent change for measurements at L-BCL and S-BCL. Dose-dependent increases in AVFRP (under all pacing conditions) and mean ventricular response during AF were noted. Drug-induced changes in AVFRP increased progressively as activation rate was increased from L-BCL to S-BCL to atrial fibrillation. Increases in mean RR interval during atrial fibrillation were, in turn, larger than the corresponding increases in AVFRP. Results shown are from four experiments after dose 1, eight experiments after dose 2, and five experiments after dose 3 of diltiazem. *p<0.05; **p<0.01; ***p<0.001 drug vs. control.

the shortest and longest pacing cycle lengths available and the changes in minimum RR interval during atrial fibrillation. After dose 1 of diltiazem, a mean increase of 10% in AVFRP over control was observed at the longest cycle length studied, whereas a 39% increase in minimum RR interval (index for AVFRP) was noted during experimental atrial fibrillation. Similar rate-dependent increases in AVFRP were observed after doses 2 and 3. Although the longest absolute values of AVFRP after drug administration were noted during pacing at the shortest pacing cycle length, larger percent increases in minimum RR interval during atrial fibrillation were observed at all doses (Table 2, Figure 4).

The relation between diltiazem’s effects on mean ventricular response and AVFRP are displayed in Figure 4. The increase in mean ventricular response produced by diltiazem during atrial fibrillation was much larger than the drug’s effect on AVFRP at long cycle lengths, and the former effect averaged 8.9, 10.9, and 9.4 times larger than the latter effect for doses 1, 2, and 3, respectively. Frequency-dependent increases in AVFRP accounted for approximately one half of the 10-fold increase in drug effect observed during atrial fibrillation (3.9–4.8 fold increases in AVFRP). The remainder of drug effect on mean ventricular response during atrial fibrillation was accounted for by an increase in concealed AV nodal conduction as reflected by an increase in the standard deviation of RR interval histograms (Table 2).

Effects of Diltiazem on Concealed Atrioventricular Nodal Conduction

The potential zone of concealment, during which nonconducted atrial impulses can penetrate the AV node and cause subsequent impulse delay or block, is defined by the difference between AERP and AVERP at any given cycle length. Figure 5 illustrates a representative experiment in which AERP and AVERP were determined at multiple cycle lengths before and after infusion of diltiazem. Before drug administration, this potential zone was small (<30 msec) at all cycle lengths tested. After diltiazem, frequency-dependent increases in AVERP, without changes in AERP were noted. Thus, the potential zone of concealment was increased by diltiazem in a frequency-dependent fashion because of increases in AVERP. A representative experiment in which this zone was scanned with interpolated atrial stimuli is shown in Figure 6. Under control conditions at a cycle length of 500 msec, AV conduction time of test stimuli was exponentially related to the test stimulus coupling interval (S,S'). After drug infusion, the relation between AV conduction time and S,S' was shifted upward and to the right. Introduction of concealed atrial extrastimuli (S') caused a further parallel horizontal shift of the AV nodal recovery curve. Earlier concealed atrial extrastimuli shifted the recovery curve to a lesser extent than did later concealed atrial extrastimuli.

In all four experiments, concealment was documented to occur in the presence of diltiazem (dose 2) at all S,S' intervals greater than the AERP and
less than the AVERP. However, the length of the zone during which atrial impulses were concealed depended on underlying heart rate, and larger zones were observed at shorter cycle lengths (Figure 7).

**Discussion**

Understanding of antiarrhythmic drug action has improved with the appreciation that cardiac frequency is an important modulator of drug action and that important differences in frequency-dependent properties exist within a specific class of drugs. These concepts have been incorporated into recent models of antiarrhythmic drug action. 

Although frequency-dependent effects on cardiac conduction and refractoriness in vivo have been documented, their importance in determining anti-arrhythmic efficacy has not been adequately addressed.

We have previously demonstrated that calcium channel blockers have important frequency-dependent effects on AV nodal conduction and refractoriness. Because atrial fibrillation results in a very fast input rate into the AV node, rapid AV nodal activation in the presence of calcium channel blockers should result in increased block of inward calcium current and a slower ventricular response rate. This would lead to enhancement of antiarrhythmic drug effects by the very tachyarrhythmias for which these drugs are used and would lead to desirable selectivity of drug action. We found that diltiazem’s actions during atrial fibrillation were disproportional to its effects at cycle lengths comparable to resting sinus rhythm. Progressive amplification by increases in atrial rate led to maximal effects during atrial fibrillation.

Although several models have been proposed to account for the ventricular response to atrial fibrillation, our results can be understood by the classic mechanisms proposed by Langendorf and extended by others. It is assumed that rapid, irregular atrial impulses penetrate the AV node with variable strength from multiple directions. The resulting ventricular response is determined by two factors: 1) the functional refractory period of the AV node, which constrains the maximum exit rate from the AV node, and 2) the role of concealed AV nodal responses. Our results suggest that the beneficial effects of diltiazem during atrial fibrillation are secondary to increases in both the AVFRP and the impairment in AV nodal conduction resulting from concealed AV nodal responses. Moreover, these effects on AV nodal properties were markedly dependent on stimulation rate, implying that frequency-dependent drug-receptor interactions may be responsible for maximizing drug effects during tachyarrhythmias.

We found that the percentage by which diltiazem increased AVFRP became progressively larger as atrial rate increased and that the largest percent increases were observed during atrial fibrillation. The rate dependence of AVFRP in the presence of diltiazem was the opposite of that described in the absence of drug (as confirmed by our control
changes in the RR interval histograms, could be due to a change in the atrial input frequency into the AV node (so that more impulses are likely to fall during zone of concealment), or to an increase in zone of concealment itself. Thiesen and coworkers\textsuperscript{14} suggested that diltiazem alters the input into the AV node during atrial fibrillation. However, diltiazem did not alter atrial refractoriness in our dogs, and in previous studies, we showed that atrial conduction is also unaffected by diltiazem.\textsuperscript{9} Because the properties of atrial fibrillation are determined by atrial conduction velocity and refractoriness and because diltiazem affects neither of these variables in autonomically blocked dogs, it is unlikely that the atrial input pattern during atrial fibrillation was altered by diltiazem in our dogs. These considerations, coupled with direct observations of zone of concealment during atrial pacing, are consistent with the hypothesis that the increases in AV nodal concealment produced by diltiazem during atrial fibrillation result from an increase in the concealment zone due to rate-dependent increases in AVERP.

Frequency-dependent increases in AV nodal refractoriness and concealment led to dramatic amplification of diltiazem’s effects during atrial fibrillation. The amplification of drug effect by tachycardia is likely related to the preferential binding of diltiazem to AV nodal calcium channels that occurs during depolarization and is followed by drug unbinding after repolarization.\textsuperscript{29} At slower rates (e.g., in sinus rhythm) diastolic time is longer, allowing more drug unbinding and less AV nodal depression. However, during atrial fibrillation, frequent AV nodal activation limits the recovery time available between impulses, leading to an accumulation of diltiazem binding and enhanced drug effects. This activation increases the AVFRP, increasing the minimum output interval that the AV node can support, and this also increases the AVERP, increasing the number of impulses that block in the AV node and that leave it in a state of increased refractoriness (concealed conduction).

**Potential Limitations**

Our model of atrial fibrillation was designed to simulate the chaotic, rapid input into the AV node that occurs during atrial fibrillation in humans. It was not intended to simulate the areas of slowed atrial conduction and increased heterogeneity of atrial refractoriness that may be responsible for spontaneous initiation and maintenance of the arrhythmia. Reservations about the applicability of observations concerning electrically induced atrial fibrillation to the spontaneous arrhythmia have been made.\textsuperscript{46} In addition, we did not obtain autocorrelograms during atrial fibrillation; therefore, periods of regularization of RR intervals may have occurred without recognition. However, the structure of RR interval histograms recorded in our experiments were unimodal and skewed to the right as previously reported in spontaneous atrial fibrillation in
humans.\textsuperscript{14,32,34,36,38} In addition, the characteristics of atrial fibrillation during continuous stimulation were similar to those of atrial fibrillation that persisted after pacing in those experiments in which the arrhythmia persisted long enough to allow analysis. Furthermore, the changes in RR interval histograms that we observed after the administration of diltiazem were very similar to those reported after oral therapy in humans.\textsuperscript{14,47}

Intravenous diltiazem does not affect intra-atrial conduction time or the HV interval.\textsuperscript{9} We have also shown that changes in the AV conduction time that occur in response to premature stimulation in this model occur exclusively as a result of changes in the AH interval, while HV times remain constant.\textsuperscript{9} Moreover, during experimental and spontaneous atrial fibrillation,\textsuperscript{17,48} all impulses activating the His bundle lead to ventricular activation, justifying the use of ventricular activation as an exit marker of the AV node. Thus, although His bundle electrograms were not used in these experiments, this does not present a major problem in interpreting the results.

These experiments were performed in autonomically blocked dogs to eliminate variability in AV conduction resulting from autonomic reflex responses to the vasodilating effects of diltiazem and to varying pacing protocols. In autonomically intact animals, we have made preliminary observations that suggest that autonomic reflexes blunt the magnitude of the effect observed at any concentration of diltiazem but that the rate dependence of drug actions are unaffected.\textsuperscript{49}

\section*{Conclusion}

In conclusion, diltiazem exerts its beneficial effects during atrial fibrillation by increasing AVFRP and the tendency of atrial impulses to manifest concealed conduction in the AV node. Both of these effects are amplified by increases in atrial rate. As a result of these frequency-dependent drug interactions, diltiazem selectively depresses AV nodal function during atrial fibrillation, and results in much smaller effects at rates corresponding to sinus rhythm in humans. This explains why doses of diltiazem in humans that have very minor effects on AV conduction during sinus rhythm can produce important and clinically useful reductions in the ventricular response rate during atrial fibrillation. These observations are consistent with the expected clinical consequences of recent models of antiarrhythmic drug action.\textsuperscript{28,29}

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