Frequency-dependent effects of calcium antagonists on atrioventricular conduction and refractoriness: demonstration and characterization in anesthetized dogs

MARIO TALAJIC, M.D., AND STANLEY NATTEL, M.D.

ABSTRACT Calcium-channel blockers are known to affect slow inward current in a frequency-dependent fashion. The purpose of these experiments was to study use-dependent effects of verapamil, diltiazem, and nifedipine on atrioventricular conduction in vivo. Loading and maintenance infusion techniques were developed to study each drug at a series of stable plasma concentrations in autonomically blocked dogs anesthetized with morphine and α-chloralose. All three agents produced changes in atrioventricular conduction and refractoriness that increased with increasing stimulation frequency. The time dependence of drug-induced changes in atrioventricular conduction was characterized both by varying the coupling of single test stimuli and by abruptly changing activation frequency. The time constants for onset of (τ_on) and recovery from (τ_off) block were typical for each drug, with nifedipine having a faster time constant (τ_off = 0.36 ± 0.12 sec) than verapamil (τ_off = 3.2 ± 1.0 sec, τ_on = 28 ± 8 sec) or diltiazem (τ_off = 2.7 ± 1.2 sec, τ_on = 13 ± 4 sec). The time constants for each drug were independent of concentration but the magnitude of time-dependent change increased with increasing drug concentration. We conclude that calcium-channel blockers have important frequency-dependent effects on atrioventricular conduction in vivo. This frequency dependence may result in selective depression of atrioventricular conduction in the presence of supraventricular tachyarrhythmias, with important potential implications for the clinical use of these agents.


ANTIARRHYTHMIC DRUGS have been shown to have important frequency-dependent properties. These were initially described in 1957 by Johnson and McKinnon, who demonstrated that quinidine's depressant effect on the maximum upstroke velocity (V_max) of ventricular fibers in vitro was more pronounced at faster driving rates. Since that time, frequency-dependent actions of sodium-channel blockers have been well described in vitro. These observations have formed the basis for different models describing the interaction between antiarrhythmic drugs and their respective receptors. Recent studies have also shown frequency-dependent effects of fast-channel blockers in vivo.

Calcium-channel blockers have also been shown to have frequency-dependent properties. Verapamil was initially found to produce a greater increase in rabbit atrioventricular (AV) nodal conduction time at faster atrial pacing rates than at slower rates, although the mechanism of this observation was unclear. Subsequently, it was observed that verapamil and its methoxy derivative, D600, had a more depressant effect on contractility as driving rate was increased. Frequency-dependent decreases in slow inward current have been demonstrated for both verapamil and diltiazem. Although nifedipine was initially reported to have a pure tonic effect on slow inward current without significant frequency dependency, more recent work with faster pacing rates has indicated that some use-dependent block occurs with nifedipine.

To date, the bulk of data on interval-dependent actions of calcium antagonists has been obtained with in vitro analysis of slow inward current. Frequency-de-
pendent depressant effects on slow-channel tissue in the AV node could have important clinical implications, if drugs selectively suppressed conduction during tachycardia but had minimal effects on AV conduction during sinus rhythm. Verapamil and diltiazem terminate reentrant supraventricular arrhythmias by depressing conduction through the AV node. A recent clinical study found rate-related slowing of AV conduction after administration of verapamil, although the time dependence of verapamil’s action was not quantitated. The purpose of our study was to characterize the frequency-dependent effects of calcium antagonists on AV nodal properties. We chose for study the three agents in current clinical use: verapamil, diltiazem, and nifedipine. A preliminary communication of these results has been presented in abstract form.

Methods

General. Mongrel dogs of either sex were anesthetized with morphine (2 mg/kg sc) and α-chloralose (100 mg/kg iv). Catheters were inserted into both femoral arteries and both femoral veins and kept patent with heparinized saline (0.9%). Dogs were ventilated with an endotracheal tube at a rate of 10/min with a tidal volume obtained from a nomogram. Arterial blood gases were measured to ensure adequate oxygenation (SaO2 ≥ 90%) and physiologic pH (7.38 to 7.45). A right thoracotomy was performed and two bipolar Teflon-coated stainless-steel electrodes were inserted into the right atrial appendage for recording and stimulation. The right atrial appendage was used in all cases to minimize the effect of atrial stimulation site on AV conduction. A Statham P23 1D transducer (Statham Medical Instruments, Los Angeles), electrophysiologic amplifiers, and a paper recorder (Grass Instruments Co. or Siemens Mingograph 80 recorder) 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 pulses at twice diastolic threshold current controlled by a programmable stimulator (Caltronics, Inc.). After autonomic blockade was performed (see below), the sinus node was crushed.

Autonomic blockade. A regimen for producing autonomic blockade was developed in four preliminary experiments. The threshold voltage for bilateral bipolar vagal stimulation with 0.2 msec square-wave pulses at 20 Hz sufficient to produce greater than 3 sec sinus pauses was determined. Atropine (1 mg iv) was given and vagal stimulation was repeated at twice the previously determined threshold voltage, with an absence of sinus slowing indicating adequate muscarinic blockade. The response to β-adrenergic stimulation was assessed by administering test doses of isoproterenol sufficient to increase heart rate by at least 35 beats/min under control conditions. Adequate β-blockade was defined as a less than 5 beat/min rise in heart rate after isoproterenol challenge. The dose of propranolol necessary to produce sustained β-blockade was found to be 0.3 mg/kg iv bolus followed by an infusion of 0.45 mg/kg/hr. A single 1 mg iv dose of atropine blocked the effects of vagal stimulation for several hours. This protocol was used for all experiments reported in this article.

Experimental protocol. Wenckebach cycle length (WBCL) was determined under control conditions by decreasing atrial pacing cycle length (BCL) by 10 msec decrements until second-degree AV block occurred. We evaluated the response of the AV node to changes in frequency in two ways. (1) Single-pulse experiments: A fixed basic cycle length (S1S2 interval) was used, and the effect of changes in coupling interval (S1S2 interval) was determined. A single premature or delayed stimulus was introduced after 20 to 40 basic stimuli (S1) and a curve relating AV conduction time (AVCT) to S1S2 interval was established. These experiments will be referred to as “single-pulse experiments.” The S1S2-AVCT relationship was determined at two basic cycle lengths. The AVCTs for basic stimuli immediately preceding the S2 were measured at all S1S2 intervals to ensure that the duration of basic stimulation before S2 was sufficient to produce a steady state. (2) Abrupt-change experiments: The response of AVCT to abrupt changes in BCL was determined. AVCT was measured at a BCL 50 msec above the WBCL. The pacing cycle length was then abruptly doubled, and AVCT was measured continually for 10 sec and then at frequent intervals for 2 min. The pacing cycle length was then abruptly halved and AVCT was measured serially as above. These experiments will be referred to as “abrupt change experiments.” AVCT was measured under steady state conditions over a wide range of BCLs (300 to 1500 msec). Steady state was achieved by pacing at a given rate for 2 min. Effective refractory period of the AV conducting system (AVERP) was measured at the same cycle lengths by the extrastimulus technique.

After the above control measurements were completed, a drug was infused intravenously and the experimental protocol was repeated. Diltiazem and verapamil were dissolved in 0.9% saline. Nifedipine was dissolved in polyethylene glycol, diluted with 0.9% saline, and shielded from light during all experiments. The total amount of polyethylene glycol used in any dog was less than 3 ml. Administration of the vehicle alone did not produce electrophysiologic changes. All drugs were obtained from Sigma Chemical Co. Dose selection was based on previously published pharmacokinetic data to produce stepwise increases in plasma concentration (table 1). Electrophysiologic study was begun 10 min after the completion of each loading dose. Blood samples for subsequent measurement of plasma drug concentrations were drawn before and after electrophysiologic study during each maintenance infusion period. Diltiazem* and nifedipine plasma concentrations were measured by previously described methods. Plasma verapamil concentrations were measured with a high-performance liquid chromatographic assay (HPLC) based on previously described methods but with norverapamil as an internal standard. Plasma propranolol concentrations were measured by HPLC in five dogs and ranged from 100 to 399 ng/ml with a mean concentration of 208 ng/ml.

Data analysis. Electrophysiologic recordings were obtained at 100 and 250 mm/sec paper speeds. AVCT was measured from the stimulus artifact to the onset of the QRS complex. In four dogs the experiments were performed with an epicardial His bundle electrode and measurements of AVCT were compared directly with the measured AH intervals. Multiple doses of nifedipine (one dog), diltiazem (one dog), and verapamil (two dogs) were evaluated in this manner. AVCT was found to be linearly related to the AH interval in each case (figure 1), with other conduction intervals remaining unaffected by the drugs studied. Accordingly, AVCT was used in all remaining experiments as an index of conduction time through the AV node. The effective refractory period (ERP) of the AV conducting system was defined as the longest S1S2 interval failing to conduct through the AV node and produce a ventricular response. Under control conditions, refractoriness of atrial tissue was sometimes limiting, and the value obtained for ERP of the

*Marion Laboratories, manuscript in preparation.
TABLE 1

Dosing regimens of calcium antagonists

<table>
<thead>
<tr>
<th>Drug concentration (ng/ml)</th>
<th>Dose</th>
<th>Loading (mg/kg)</th>
<th>Maintenance (mg/kg/min)</th>
<th>Prestudy</th>
<th>MeanA</th>
<th>Poststudy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verapamil</td>
<td>Dose 1</td>
<td>0.05</td>
<td>0.00025</td>
<td>23 ± 8</td>
<td>18 ± 3</td>
<td>11 ± 1</td>
</tr>
<tr>
<td></td>
<td>Dose 2</td>
<td>0.05</td>
<td>0.00025</td>
<td>26 ± 21</td>
<td>37 ± 30</td>
<td>34 ± 30</td>
</tr>
<tr>
<td></td>
<td>Dose 3</td>
<td>0.10</td>
<td>0.0005</td>
<td>75 ± 34</td>
<td>73 ± 28</td>
<td>72 ± 56</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>Dose 1</td>
<td>0.1</td>
<td>0.001</td>
<td>36 ± 17</td>
<td>42 ± 23</td>
<td>42 ± 23</td>
</tr>
<tr>
<td></td>
<td>Dose 2</td>
<td>0.2</td>
<td>0.003</td>
<td>71 ± 46</td>
<td>83 ± 40</td>
<td>101 ± 35</td>
</tr>
<tr>
<td></td>
<td>Dose 3</td>
<td>0.4</td>
<td>0.007</td>
<td>191 ± 64</td>
<td>203 ± 60</td>
<td>223 ± 73</td>
</tr>
<tr>
<td></td>
<td>Dose 4</td>
<td>0.8</td>
<td>0.015</td>
<td>427 ± 207</td>
<td>467 ± 176</td>
<td>528 ± 170</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>Dose 1</td>
<td>0.2</td>
<td>0.002</td>
<td>89 ± 56</td>
<td>75 ± 39</td>
<td>62 ± 31</td>
</tr>
<tr>
<td></td>
<td>Dose 2</td>
<td>0.4</td>
<td>0.004</td>
<td>249 ± 107</td>
<td>226 ± 75</td>
<td>184 ± 78</td>
</tr>
</tbody>
</table>

Electrophysiologic studies were begun 10 min after the end of loading infusions. Diltiazem and verapamil loading doses were infused over 10 min. Nifedipine loading doses were infused over 20 min.

A Mean values were calculated from experiments from which both prestudy and poststudy values were available. Because of data dropout, some overall mean values shown are higher than mean prestudy or poststudy values displayed.

AV conducting system was an upper limit for the AV nodal ERP. In the presence of significant concentrations of calcium antagonists, the AV node ERP always exceeded atrial ERP and was directly reflected by the refractory period of the AV conducting system.

Group data are presented in this manuscript as mean ± SD. Multiple comparisons between control and experimental group means were made by two-way analysis of variance with Scheffé's test.32 Comparisons between two groups of experimental data were made with unpaired t tests. Two-tailed tests were used for all statistical comparisons and a probability of 5% or less was taken to indicate statistical significance.

Results

Hemodynamic and electrophysiologic variables. Diltiazem, verapamil, and nifedipine increased AVCT, WBCL, and AV effective refractory period (AVERP) in a dose-dependent manner (table 2). For a given plasma concentration, verapamil had the greatest effect on AV nodal properties, followed by diltiazem and then nifedipine.

Systolic and diastolic blood pressure were significantly reduced by all three drugs. The dose range of nifedipine that could be studied was limited by hypotension at doses above those shown in table 1. Plasma drug concentrations were relatively constant over the duration of each infusion, and WBCL measured at the beginning and end of each maintenance infusion varied by less than 10% in all experiments.

Single-pulse experiments. Examples of three experiments relating AVCT to S1S2 interval under control conditions and after drug infusion are shown in figure 2. Under control conditions AVCT was constant over a wide range of coupling intervals greater than 500 msec. With further decreases in coupling interval, there was a rapid increase in AVCT until refractoriness was encountered. Changes in AVCT compared with the AVCT of the longest coupled beat (ΔAVCT) had an exponential relationship with S1S2 interval (time constant averaged 81 ± 24 msec; n = 28). In the presence of all three calcium antagonists, AVCT was prolonged and the relationship between ΔAVCT and S1S2 became biexponential (figure 2). For verapamil and diltiazem, recovery continued to occur at coupling intervals of up to 10 sec, whereas with nifedipine no further recovery occurred for activations with coupling intervals greater than 1500 msec.

Quantification of the time constants for the fast phase (seen both for control and drug infusion periods) and for the slow phase (seen only in the presence of calcium antagonists) was carried out with a nonlinear curve fitting technique and is summarized in table 3. The fast phase time constants (t-fast) for all three drugs were not significantly different from their respective control values. The mean slow phase time constant for nifedipine (357 ± 117 msec) was significantly shorter than the mean time constants for diltiazem (2.7 ± 1.2 sec; p < .001) or verapamil (3.2 ± 1.0 sec; p < .001). The mean slow-phase time constants were not concentration dependent. There was no significant difference between the mean slow-phase time constants for diltiazem and verapamil. The maximum increase in AVCT that occurred just before the
refractory period (total $\Delta$AVCT) was not altered by any drug infusion. However, the magnitude of change in AVCT that occurred during the slow phase ($\Delta$AVCT-slow) was concentration dependent for all three drugs. There were no significant differences between the maximum pauses that could be studied under control conditions and those seen after drug infusion.

**Abrupt-change experiments.** Under control conditions an abrupt change in pacing cycle length caused a rapid change in AVCT for the first beat at the new driving rate. After the first beat, there was minimal further change in AVCT (always less than 12 msec) over the next 2 min.

With infusion of diltiazem or verapamil, the curve of AVCT vs time after changing frequency (figure 3, bottom) was altered by the appearance of a substantial slower change in AVCT. Steady-state AVCT was reached after 20 to 30 sec with diltiazem and 60 to 80 sec with verapamil. Drug-induced changes in AVCT after the first beat at the new pacing rate were well fitted by a logarithmic function of time (figure 3, top).

Table 4 summarizes the results of quantification of the magnitude and time course of the changes in AVCT. The mean time constant of change in AVCT for diltiazem was $13.0 \pm 3.9$ sec, which was significantly different from the time constant measured for verapamil ($28.0 \pm 7.7$ sec; $p < .001$). The time constants after decreases in rate were similar to those ob-

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**FIGURE 1.** AVCT, AH, HV, and SA intervals as a function of coupling interval after administration of verapamil (0.2 mg/kg iv load and 0.005 mg/kg/min maintenance infusion). The right atrium was stimulated with a series of basic ($S_1$) stimuli, followed by a test stimulus ($S_2$) with a selected ($S_1S_2$) coupling interval. AVCT was measured from the stimulus artifact to the onset of the QRS complex. SA, AH, and HV intervals were measured using the stimulus artifact ($S$) and the His bundle electrogram. Changes in AH interval were reflected directly by corresponding changes in AVCT, whereas SA and HV intervals were constant over the range of coupling intervals examined. Inset, AH interval plotted as a function of AVCT in the same experiment. AH was found to be linearly related to AVCT over the range of coupling intervals studied. The equation of best fit by linear regression was AVCT = $76 + 1.02$ (AH), $r = .999$. In two experiments studying multiple infusions of verapamil, one studying diltiazem, and one studying nifedipine, all frequency-dependent changes in AVCT were found to be attributable to changes in AH interval.
TABLE 2
Hemodynamic and electrophysiologic variables before and after infusion of calcium antagonists

<table>
<thead>
<tr>
<th>Conc. (ng/ml)</th>
<th>AVCT (msec)</th>
<th>ERP (msec)</th>
<th>WBCL (msec)</th>
<th>Blood pressure (mm Hg)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Systolic</td>
</tr>
<tr>
<td>Verapamil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>130 ± 16</td>
<td>165 ± 11</td>
<td>241 ± 16</td>
<td>119 ± 14</td>
</tr>
<tr>
<td>18 ± 3</td>
<td>142 ± 11</td>
<td>214 ± 37</td>
<td>296 ± 35</td>
<td>114 ± 13</td>
</tr>
<tr>
<td>37 ± 30A</td>
<td>155 ± 9C</td>
<td>283 ± 109C</td>
<td>359 ± 68</td>
<td>108 ± 15B</td>
</tr>
<tr>
<td>73 ± 28A</td>
<td>188 ± 24D</td>
<td>503 ± 345C</td>
<td>563 ± 289D</td>
<td>103 ± 15D</td>
</tr>
<tr>
<td>Diltiazem</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>118 ± 16</td>
<td>142 ± 26</td>
<td>224 ± 25</td>
<td>123 ± 13</td>
</tr>
<tr>
<td>42 ± 23</td>
<td>129 ± 11</td>
<td>161 ± 35</td>
<td>252 ± 34</td>
<td>118 ± 14</td>
</tr>
<tr>
<td>83 ± 40</td>
<td>158 ± 15D</td>
<td>245 ± 50</td>
<td>336 ± 47C</td>
<td>119 ± 16</td>
</tr>
<tr>
<td>203 ± 60A</td>
<td>197 ± 28D</td>
<td>382 ± 128D</td>
<td>471 ± 113D</td>
<td>116 ± 14</td>
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<td>467 ± 176A</td>
<td>248 ± 34D</td>
<td>557 ± 147D</td>
<td>651 ± 131D</td>
<td>109 ± 19B</td>
</tr>
<tr>
<td>Nifedipine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>134 ± 3</td>
<td>134 ± 15</td>
<td>208 ± 11</td>
<td>139 ± 8</td>
</tr>
<tr>
<td>75 ± 39</td>
<td>137 ± 11</td>
<td>133 ± 18</td>
<td>268 ± 33B</td>
<td>116 ± 14D</td>
</tr>
<tr>
<td>226 ± 75</td>
<td>154 ± 23C</td>
<td>184 ± 66B</td>
<td>346 ± 60D</td>
<td>101 ± 9D</td>
</tr>
</tbody>
</table>

All measurements were obtained at a BCL of 600 msec. Results shown are for 14 experiments with diltiazem, and seven each with verapamil and nifedipine. Numbers are reported as mean ± SD. Each set of mean data shown is for all experiments at a given dose with a given drug. For doses used to produce the mean concentrations shown, see table 1.

Conc. = mean plasma concentration at the time of electrophysiologic study.

Data from several experiments could not be obtained at BCL 600 because of AV block at this cycle length; data shown are for the shortest BCL studied above WBCL for experiments in which WBCL was ≥600 msec.

*p < .05; ′p < .01; ″p < .001, all vs control.

FIGURE 2. Relationship between AV conduction and coupling interval before and after infusion of verapamil (left), diltiazem (center), and nifedipine (right) in representative experiments. The right atrium was stimulated with a series of basic (S1) stimuli, followed by a test stimulus (S2) with a selected (S1,S2) coupling interval. Differences in AV conduction time (ΔAVCT) between AVCT at each S1,S2 interval and AVCT at the longest S1,S2 interval obtained were plotted as a function of S1,S2 interval. Under control conditions, ΔAVCT was always a single exponential function of S1,S2 interval with a time constant averaging 81 msec. In the presence of calcium antagonists, the relationship became biexponential with a rapid component indistinguishable from control and a slower component of interval-dependent recovery. The dashed lines in the graphs are curves fitted to the observed values by either a single-exponential model (control) or a biexponential model (in the presence of drugs). The time constant of the slow phase for all experiments averaged 3.2 sec for verapamil, 2.7 sec for diltiazem, and 0.36 sec for nifedipine. The rate of the slow phase was not concentration-related, but its magnitude increased with increasing drug concentration.
The magnitude of change of AVCT that occurred after the first beat (ΔAV-slow) was concentration dependent. For nifedipine, most of the alteration in AVCT was achieved in the first 1 to 3 beats after a change in stimulation rate and no clear slow phase of AVCT adaptation was observed (figure 3).

**Drug effects on AV conduction and refractoriness: dependence on BCL.** Under control conditions AVCT increased at short pacing cycle lengths (figure 4). The magnitude of change from the shortest to the longest AVCT averaged 32 ± 13 msec under control conditions, and the range of cycle lengths over which changes in AVCT occurred averaged 161 ± 98 msec. The magnitude of change in AVCT with changes in activation rate, and the range of BCLs over which changes in AVCT occurred were increased by calcium antagonists in a dose-dependent fashion (figure 4, table 5). The largest changes were seen with diltiazem, followed by verapamil and then nifedipine.

Under control conditions, the effective refractory period of the AV conducting system decreased slightly with decreasing cycle length (figure 5). In the presence of calcium antagonists, there was a dose-related increase in AVERP with decreasing cycle length. In addition, the range of cycle lengths over which changes in refractory period occurred increased with increasing drug dose (table 5).

**Discussion**

We have demonstrated that the calcium-channel blockers in current clinical use have important frequency-dependent effects on AV nodal conduction and refractoriness. Changes in conduction with varying coupling intervals have been characterized in a model that allows for clear separation between the intrinsic time-dependent properties of the AV node and slower, superimposed changes resulting from calcium antagonists. Under control conditions, changes in AV nodal conduction resulting from premature stimuli were well described by a single exponential with a mean time constant of about 80 msec. This agrees well with previous observations of the interval dependence of AV nodal conduction in vivo33, 34 and is in the same range as the time constant for slow current recovery in multicellular preparations. 35 In the presence of calcium antagonists, an additional slower phase of recovery of AVCT was noted with pauses of increasing duration, presumably due to recovery of drug-associated chan-
nels. The time course of this phase depended on the drug studied and appeared to be concentration independent, whereas its magnitude increased with increasing drug concentration. Accumulation and reduction of drug effect upon abrupt changes in stimulation frequency also followed an exponential time course typical for each drug studied, with a time constant that was concentration independent and a magnitude that was related to concentration.

Our results can be understood in terms of recent

**FIGURE 3.** Relationship between AV conduction and time after an abrupt increase in heart rate before and after infusion of verapamil (left), diltiazem (center), and nifedipine (right) in representative experiments. The right atrium was stimulated with a series of basic (S1) stimuli for 2 min and then the rate was abruptly doubled. **Top.** Differences in AVCT (Δ to steady-state AVCT) between AVCT at each time point and the new steady-state AVCT, plotted as a function of time after the change in rate. Under control conditions, most of the change in AVCT occurred with the first beat after the change in rate with minimal further change over the next 2 min. In the presence of diltiazem or verapamil, a slower phase of change in AVCT occurred after the first beat at the new rate. In both cases, the time course of this slower phase was fitted with a single exponential. The dashed lines in the upper panels are fitted to the observed values by least-squares regression. The mean time constant in all experiments averaged 13.0 sec for diltiazem and 28.0 sec for verapamil. The rate of the slow phase was not related to concentration, but its magnitude increased with increasing drug concentration. No consistent quantifiable slow phase of AVCT change was seen after abrupt alteration in rate in experiments studying nifedipine.
TABLE 4
Abrupt-change experiments: magnitude and time course of changes in AVCT\(^a\)

<table>
<thead>
<tr>
<th>Conc. (ng/ml)</th>
<th>Tau (sec)</th>
<th>(\Delta)AV-slow (msec)</th>
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</thead>
<tbody>
<tr>
<td>Verapamil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 ± 3</td>
<td>28.0 ± 6.0</td>
<td>.95 ± .02</td>
</tr>
<tr>
<td>37 ± 30</td>
<td>32.0 ± 6.0</td>
<td>.96 ± .04</td>
</tr>
<tr>
<td>73 ± 28</td>
<td>23.9 ± 7.2</td>
<td>.99 ± .01</td>
</tr>
<tr>
<td>Diltiazem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42 ± 23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>83 ± 40</td>
<td>11.4 ± 2.6</td>
<td>.98 ± .01</td>
</tr>
<tr>
<td>203 ± 60</td>
<td>13.5 ± 3.1</td>
<td>.99 ± .01</td>
</tr>
<tr>
<td>467 ± 176</td>
<td>10.8 ± 2.4</td>
<td>.98 ± .01</td>
</tr>
</tbody>
</table>

\(^a\)Tau is the time constant of change in AVCT after the first beat from the change in stimulation rate. \(\Delta\)AV-slow is a reflection of the magnitude of the slow process of change in AVCT at that drug concentration. Changes in AVCT (\(\Delta\)AVCT) after abrupt rate alteration were fitted to an equation of the form \(\Delta\)AVCT = (\(\Delta\)AV-slow)\(\cdot\)e\(^{-t/\tau}\) where \(t = \) time since the change in pacing cycle length and \(\Delta\)AVCT = difference between AVCT at time \(t\) and the steady-state AVCT 2 min after altering pacing rate. Conc. = mean plasma drug concentration at the time of electrophysiologic study. Values are reported as mean ± SD. Results shown are for five diltiazem and five verapamil experiments.

Changes during the slow phase were too small to calculate tau accurately in enough dogs to obtain a meaningful mean value. Each set of mean data shown is for all experiments at a given dose with a given drug. For doses used to produce the mean concentrations shown, see Table 1.

models of antiarrhythmic drug action.\(^2,6\) The single-pulse recovery data reflect the recovery of calcium channels from depression by calcium antagonists, presumably via diastolic dissociation of drugs from their receptor sites. Abrupt alterations in basic frequency result in a more complex process, including changes both in drug dissociation between beats and in drug association occurring with successive activations.\(^4,6\) It would therefore not be surprising to find, as we did, that the rate of alteration in drug effect measured by varying the coupling interval of single pulses (single-pulse experiments) is different from the time course observed upon changing basic stimulation rates (abrupt-change experiments). Furthermore, accurate quantitation of time constants requires that a process be followed until steady-state change is observed. With abrupt changes in frequency, observations clearly can be (and were) carried out until steady-state conditions occur. For single-pulse experiments, however, the maximum pauses that can be studied are limited by spontaneous junctional escape beats and by the physiologic requirements of a system in vivo. In the case of nifedipine, maximal time-dependent recovery was attained well within the range of pause durations available. For diltiazem and verapamil, however, even with
<table>
<thead>
<tr>
<th></th>
<th>Changes in AVCT</th>
<th>Changes in ERP</th>
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<tbody>
<tr>
<td></td>
<td>Conc. (ng/ml)</td>
<td>ΔAVCT (msec)</td>
</tr>
<tr>
<td>Verapamil</td>
<td>Control</td>
<td>35 ± 9</td>
</tr>
<tr>
<td></td>
<td>18 ± 3</td>
<td>42 ± 9</td>
</tr>
<tr>
<td></td>
<td>37 ± 30</td>
<td>49 ± 11</td>
</tr>
<tr>
<td></td>
<td>73 ± 28</td>
<td>56 ± 7</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>Control</td>
<td>34 ± 14</td>
</tr>
<tr>
<td></td>
<td>42 ± 23</td>
<td>45 ± 20</td>
</tr>
<tr>
<td></td>
<td>83 ± 40&lt;sup&gt;A&lt;/sup&gt;</td>
<td>55 ± 11&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>203 ± 60</td>
<td>58 ± 17&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>467 ± 176</td>
<td>74 ± 21&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>Control</td>
<td>25 ± 14</td>
</tr>
<tr>
<td></td>
<td>75 ± 39</td>
<td>35 ± 22</td>
</tr>
<tr>
<td></td>
<td>226 ± 75&lt;sup&gt;C&lt;/sup&gt;</td>
<td>39 ± 17&lt;sup&gt;C&lt;/sup&gt;</td>
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</tbody>
</table>

Results shown are for 10 experiments with diltiazem and seven each with verapamil and nifedipine. Each set of mean data shown is for all experiments at a given dose with a given drug. For doses used to produce the mean concentrations shown, see table 1.

ΔAVCT = total observed change in AVCT from the shortest to the longest cycle length studied; ΔERP = total observed change in effective refractory period of the AV conducting system from shortest to longest cycle length studied (a positive number implies an increase in ERP with shorter cycle lengths); BCL range = range of cycle lengths over which greater than 10 msec changes were observed relative to the AVCT or ERP recorded at the longest cycle length studied. Conc. = mean plasma drug concentration at the time of electrophysiologic study.

<sup>A</sup>p ≤ .05; <sup>B</sup>p ≤ .01; <sup>C</sup>p ≤ .001, all vs control.

the longest pauses that could be produced in single-pulse experiments, continued recovery of AV conduction was observed. Thus, although our single-pulse experiments clearly show a slow, time-dependent recovery from diltiazem- and verapamil-induced blockade, the calculated time constants may not precisely reflect the recovery rate of drug-associated calcium channels.

Nevertheless, our estimates are in general agreement with previously published work on the effects of calcium blockers on inward calcium current in vitro. Uehara and Hume<sup>36</sup> described a recovery time constant of 2.2 sec for diltiazem, which is similar to our estimate of 2.7 sec. Tung and Morad<sup>16</sup> reported reestablishment of inward calcium current block within 25 sec in the presence of diltiazem, in comparison with our observation that diltiazem’s maximal effect on AVCT took 20 to 30 sec to develop after an abrupt change in frequency. The time to achieve a new steady-state AVCT after an abrupt change in rate (60 to 80 sec) after infusion of verapamil is of the same order of magnitude reported in previous studies in vitro.<sup>14, 19</sup>

We found a significant tonic effect of nifedipine on AV conduction, as well as some frequency dependence at very short cycle lengths and coupling intervals. Uehara and Hume<sup>36</sup> reported that most of the slow channel blockade produced by nifedipine in single frog

atrial cells was tonic. They observed a small use-dependent portion having a mean time constant of 493 msec, and in our studies the slow time constant of recovery of AVCT in the presence of nifedipine averaged 357 msec. We could not demonstrate a frequency-dependent change in AVCT by nifedipine using abrupt doublings or halvings of rate, but the frequencies of stimulation that could be studied would be unable to resolve a time constant much below 1 sec. The current data also suggest that the limited effect of nifedipine on AV nodal properties in vivo is caused not only by the described reflex autonomic changes but also by rapid recovery of the AV node from nifedipine-induced depression. In addition, the potent hypotensive effects of nifedipine limit the dose range that can be safely used.

The degree to which the time dependence of drug-induced conduction changes in vivo parallel effects on the corresponding inward current(s) in vitro is as yet unclear. In canine cardiac Purkinje fibers, there is a close relationship between recovery time constants for effects on V<sub>max</sub> and conduction.<sup>37</sup> The interval dependence of amitriptyline-induced QRS prolongation in vivo parallels the time course of changes in V<sub>max</sub> in vitro.<sup>9</sup> Preliminary work has been published suggesting that the time course of changes in conduction velocity produced by mexiletine in vivo may be more
rapid than for changes in $V_{\text{max}}$ in vitro.\textsuperscript{38} However, the time constant for mexiletine’s effects on conduction in vivo in the latter study was similar to time constants measured by other investigators for the drug’s effect on conduction\textsuperscript{37} and $V_{\text{max}}$\textsuperscript{37,39} in vitro. The precise relationship between the time dependence of measures of inward current and conduction in the presence of antiarrhythmic drugs is still uncertain. Nonetheless, there is considerable evidence that, at least for drugs that alter sodium conductance, the frequency dependence of changes in measures of inward current is closely related to use-dependent changes in conduction. Changes in passive membrane properties and the voltage dependency of recovery rate\textsuperscript{3,13,14,40,41} could effect the relationship of drug-induced alterations in inward currents studied in vitro to conduction changes in vivo. The present experiments would not define voltage-dependent differences in drug-receptor binding and unbinding.

We measured total AV conduction time rather than a more direct index of AV nodal conduction such as the AH interval. However, we found that AVCT was linearly related to AH interval in the absence and presence of calcium antagonists. In addition, clinical doses of calcium antagonists (producing drug concentrations similar to those we studied in dogs) have not been found to alter intra-atrial conduction or the HV interval.\textsuperscript{20,23,42} Autonomic blockade was used to eliminate variability in AV conduction resulting from autonomic reflex responses to the vasodilating effects of calcium antagonists and to varying pacing protocols. The concentrations of propranolol achieved are within the range reported to produce $\beta$-blockade without local anesthetic effects.\textsuperscript{43} The degree to which intact autonomic tone would modify the observed changes is unknown, but qualitatively similar effects on AH interval resulting from verapamil in the absence of autonomic blockade have been reported.\textsuperscript{23} The plasma concentration range of calcium antagonists that we studied parallels the clinical range reported for each agent.\textsuperscript{44-46}

It is likely that the time-dependent changes in AV nodal conduction we have observed in the presence of calcium-channel blockers are caused by preferential drug binding during depolarization followed by drug unbinding after repolarization.\textsuperscript{4,6} With prolonged pauses (or at slower heart rates) there is more time for drug unbinding between activations, and conduction slowing is reduced. At rapid heart rates, the limited

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5}
\caption{Relationship between the effective refractory period (ERP) of the AV conducting system and steady-state pacing frequency before and after infusion of verapamil (left), diltiazem (center), and nifedipine (right). The right atrium was paced with a series of basic (S1) stimuli for greater than 2 min at each rate, after which the ERP of the AV conducting system was determined. A slight decrease in ERP occurred at shorter cycle lengths under control conditions. In the presence of calcium antagonists, AV nodal ERP increased with decreasing cycle length. The range of cycle lengths over which changes in ERP were observed and the total change in ERP over that cycle length range were both dose dependent (table 5).}
\end{figure}
recovery time between beats leads to an accumulation of drug-associated channels and a corresponding enhancement of effects on AV nodal conduction and refractoriness. These observations have important potential relevance to the clinical use of calcium antagonists. By greatly prolonging refractoriness of the AV node at the rapid frequencies (150 to 250/min) seen with reentrant supraventricular tachycardias, verapamil or diltiazem can interrupt the reentrant circuit within the AV node. Upon conversion to sinus rhythm at a slower rate, these agents produce much less depression of AV node conduction. Similarly, when the AV node is being activated frequently in the presence of atrial fibrillation or flutter, there will be substantial association of diltiazem or verapamil with calcium channels, resulting in increased AV nodal refractoriness and a reduced ventricular response rate.\(^4,\)\(^5\) The latter effect may well be seen at concentrations that cause little or no observable depression of AV conduction at normal heart rates. Investigators have speculated that the frequency dependence of antiarrhythmic drug action could selectively prevent or terminate tachycardias with little depressant effect on conduction of normal rhythms.\(^4\) Our results suggest that the effects of calcium antagonists may well fulfill this type of predicted behavior.

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Frequency-dependent effects of calcium antagonists on atrioventricular conduction and refractoriness: demonstration and characterization in anesthetized dogs.

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