The T-Type Ca\textsuperscript{2+} Channel Blocker Mibefradil Prevents the Development of a Substrate for Atrial Fibrillation by Tachycardia-Induced Atrial Remodeling in Dogs

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**Background**—Ca\textsuperscript{2+} overload is believed to play a role in tachycardia-induced atrial electrophysiological remodeling. L-type Ca\textsuperscript{2+} channel blockers attenuate effective refractory period (ERP) changes caused by 24 hours of atrial tachycardia but may not substantially alter atrial fibrillation (AF) inducibility. This study assessed the effects of the T-type Ca\textsuperscript{2+} channel blocker mibefradil on tachycardia-induced atrial remodeling.

**Methods and Results**—Dogs subjected to rapid atrial pacing (400 bpm) for 7 days were treated with mibefradil (100 mg/d, n = 8) or matching placebo (n = 10) in blinded fashion. Radiofrequency ablation of atrioventricular conduction and ventricular pacing were used to control ventricular rate. Placebo dogs showed significant decreases in atrial ERP (76 ± 5 ms at a cycle length of 300 ms) and increases in ERP heterogeneity (27.7 ± 2.4%), AF duration (414 ± 232 seconds), and AF inducibility by single extrastimuli (41 ± 10% of sites) compared with 10 unpaced control dogs (ERP 114 ± 3 ms, ERP heterogeneity 13.8 ± 0.9%, AF duration 7 ± 3 seconds, AF inducibility 1.9 ± 1.0% of sites). The changes caused by atrial tachycardia were strongly attenuated in mibefradil dogs, with ERPs averaging 102 ± 7 ms, ERP heterogeneity 18.8 ± 1.4%, AF duration 3 ± 1 seconds, and AF inducibility 9.6 ± 4.0% of sites. Among mibefradil-treated dogs, ERP, AF duration, and inducibility correlated with plasma drug concentration. Acute mibefradil administration did not alter ERP or AF.

**Conclusions**—Mibefradil, a drug with strong T-type Ca\textsuperscript{2+} channel blocking properties, prevents AF-promoting electrophysiological remodeling by atrial tachycardia. These findings have important potential implications for the mechanisms of tachycardia-induced atrial remodeling and demonstrate the feasibility of preventing electrical remodeling caused by several days of atrial tachycardia. (*Circulation*. 1999;100:2191-2197.)

Key Words: arrhythmia ■ antiarrhythmia agents ■ electrophysiology ■ calcium channels

Atrial fibrillation (AF) is the most frequently encountered arrhythmia in clinical practice\textsuperscript{1,2} and is associated with an increased mortality rate.\textsuperscript{3} Moreover, AF remains a therapeutic challenge, in part because of limitations in our understanding of the pathological and electrophysiological mechanisms underlying the arrhythmia.\textsuperscript{4} Epidemiological studies have shown that paroxysmal AF can progress to chronic AF in the absence of underlying structural heart disease.\textsuperscript{5} Moreover, the success rate of cardioversion and maintenance of sinus rhythm thereafter is also related to the duration of the arrhythmia. These observations suggest that AF is a progressive disease and has a self-perpetuating nature. Over the past few years, rapid atrial activation has been shown to increase the vulnerability to AF induction by premature extrastimuli and to promote the maintenance of AF.\textsuperscript{6-13} AF models based on tachycardia-induced remodeling are relevant to the process by which AF alters atrial electrophysiology to favor its own maintenance (“AF begets AF”).\textsuperscript{8} There is evidence that Ca\textsuperscript{2+} overload may be important in tachycardia-induced remodeling.\textsuperscript{10} Acute administration of the L-type Ca\textsuperscript{2+} current (\(I_{\text{CaL}}\)) blocker verapamil has been found to prevent effective refractory period (ERP) reduction and AF promotion by very short episodes of burst pacing–induced AF (\(\approx 10\) minutes) in humans.\textsuperscript{14,15} Verapamil was also found to attenuate changes in atrial ERP caused by 24 hours of atrial tachycardia in goats; however, AF inducibility remained high (34% in the presence of verapamil versus 39% in its absence).\textsuperscript{16} In previous studies, we noted that atrial \(I_{\text{CaL}}\) was downregulated by sustained rapid atrial pacing in dogs, whereas T-type Ca\textsuperscript{2+} current (\(I_{\text{CaT}}\)) was not reduced.\textsuperscript{17} We speculated that because \(I_{\text{CaT}}\) is not reduced even after 6 weeks of atrial tachycardia, in contrast to \(I_{\text{CaL}}\), which is decreased by \(\approx 70\%\), T-type current may provide a continuing leak of Ca\textsuperscript{2+} into the cell. \(I_{\text{CaT}}\) inhibition may therefore be necessary to prevent electrical

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remodeling caused by sustained atrial tachycardia. We therefore compared the effects of mibefradil, a selective ICa blocker, with those of identical-appearing placebo tablets on tachycardia-induced atrial remodeling in dogs.

Methods

Pacemaker Implantation and AV Junction Ablation

Adult mongrel dogs (26.7±0.2 kg, n=18) were anesthetized with sodium pentobarbital (30 mg/kg IV, additional doses of 4 mg/kg as needed). Tined unipolar pacing leads were inserted in the right atrial appendage (RAA) and in the right ventricular apex under fluoroscopic guidance. Subcutaneous pacemakers were implanted in the left anterior (atrial pacemaker) and right posterior (ventricular pacemaker) sides of the neck and connected to the appropriate pacing leads. AV block was created with radiofrequency energy (30 to 40 W for 20 to 30 seconds). The average number of radiofrequency applications was 2±0.3 (range, 1 to 5). No dogs recovered AV nodal conduction during the study. The ventricular demand (VVIP) pacemaker was programmed to capture the ventricles at 80 bpm, and the atrial pacemaker was activated to pace the atria at 400 bpm (pulse amplitude 3 times diastolic threshold). Atrial and ventricular pacing were applied continuously during the 7-day rapid atrial pacing period before electrophysiological study. Figure 1 shows a radiograph during pacemaker implantation and AV node ablation as well as a typical ECG after the procedure.

Experimental Protocol

In rapidly paced dogs, one 100-mg tablet/d of mibefradil (n=8 dogs) or matching placebo (n=10) was given in a blinded fashion beginning 4 days before pacemaker implantation and continuing until 24 hours before the day of electrophysiological study (Figure 2). Blood samples were obtained before anesthesia on study days for subsequent measurement of plasma mibefradil concentration by high-performance liquid chromatography. A group of size-matched dogs (n=10) without pacemaker implantation was used as a control group.

On the study day, dogs were anesthetized with morphine (2 mg/kg SC) and oral chloralose (120 mg/kg IV load, 29.3 mg·kg−1·h−1). The surface ECG was recorded to confirm maintained atrial and ventricular pacing and AV block. The atrial pacemaker was then deactivated and a median sternotomy performed. The study preparation and instrumentation were as previously described in detail. A mapping system was connected to 5 arrays covering the atrial epicardial surfaces with 240 bipolar electrodes (Figure 3) as previously described. ERP and conduction velocity (CV) were measured during stimulation at sites in various atrial regions as in previous work. Activation maps for CV measurement were obtained after 60 seconds at a basic cycle length of 300 ms. CV was measured with the use of 2 parallel sets of electrodes (4 bipolar electrodes per set) during local pacing in each of 5 regions: Bachmann's bundle, the left atrial appendage, the RAA, the right superior free wall, and the right inferior free wall (Figure 3). ERP was determined at an average of 15±1 sites in the same regions as for CV measurements, allowing for the calculation of local wavelength as the product of mean local CV and ERP. Comparable numbers of sites were studied in each region for each dog to avoid introducing potential sources of bias. A 15-stimulus basic train at a basic cycle length (S1S1, 2-ms, twice-threshold current pulses) of 300 ms was followed by a premature extrastimulus (S2) at a progressively increasing S1S2 interval and a 2-second pause to observe the response between trains. The coupling interval of S2 was increased by 10-ms increments to obtain an initial estimate of the ERP. The measurement was then repeated with 5-ms increments, and the resulting value was taken as the ERP. In the case of a ≥10-ms difference between the 2 measurements, a third measurement with 5-ms steps was obtained, and the mean of all 3 ERP values was used.

AF vulnerability was determined by evaluating the response to single S2s at coupling intervals of 5 and 10 ms longer than the ERP at each site used for ERP determination. The vulnerability to AF induction at each site was defined by the ability of single S2s to induce, in a reproducible fashion, AF that lasted >1 second. Overall vulnerability in each dog was defined as the percentage of pacing sites at which AF was inducible. Because AF was not inducible by single extrastimuli in all dogs, AF was also induced by stimulating the RAA with 10-Hz, 2-ms stimuli at 4 times threshold current for 2 to 10 seconds. To calculate mean AF duration, AF was induced with burst pacing 10 times for AF duration <10 minutes and twice for AF duration >10 minutes. AF that lasted >30 minutes was terminated by DC electrical cardioversion, and 20 minutes was allowed before AF induction was repeated.

Data Analysis

The CV was determined in each region as previously described, and the overall CV for each dog was calculated from the average of each of the 5 regional CV values. Overall wavelength was calculated as the mean of all ERP values in each dog times the mean CV. The overall wavelength calculated in this fashion was not significantly different from the value obtained by multiplying the mean ERP in each region by local CV and averaging the values. The coefficient of variance in ERP was calculated as SD/mean×100% and used as an index of ERP heterogeneity. The number of sites for ERP determination in each region was equivalent across dogs and between groups, to prevent any selection bias. Statistical comparisons be-

![Figure 1](image-url)
between 2 groups only were performed by Student’s t test or the Mann-Whitney rank sum test when a normal distribution could not be assumed. ANOVA (for parametric data) or a Kruskal-Wallis rank sum test was used for multiple-group comparisons, followed by a Bonferroni-corrected t test or a corrected Dunnett rank sum test. Pearson correlation was used to assess the correlation between a dependent and independent variable, and a x² test was used for contingency comparisons. Average results are given as the mean±SEM, and a 2-tailed P<0.05 was considered statistically significant.

Results

Overall Electrophysiological Changes

Control, placebo, and mibefradil dogs were similar in terms of size, number of sites for ERP determination, and atrial diastolic threshold (Table). Mibefradil-treated dogs had a slower sinus rate, consistent with the significant role of T-type Ca²⁺ channels in sinus node automaticity.²⁰ Consistent with previous observations,⁶–¹⁰,¹²,¹³ placebo-treated dogs subjected to 7 days of rapid atrial pacing had significantly increased vulnerability to AF induction and AF duration, along with reduced ERP and wavelength and increased ERP variability. Mibefradil strongly attenuated these effects of rapid atrial pacing, resulting in significantly reduced atrial vulnerability, AF duration, and ERP heterogeneity, along with increased mean ERP and wavelength, compared with placebo dogs. For mibefradil-treated rapidly paced dogs, the only electrophysiological variable that was significantly different from control (nonpaced) dogs was ERP heterogeneity: AF duration, vulnerability, mean ERP, and wavelength were not significantly altered.

Figure 4 shows representative examples of arrhythmias induced by atrial extrastimuli at sites with different refractory periods in placebo dogs (top) and mibefradil dogs (bottom). At sites with longer ERPs in placebo dogs (A), arrhythmias were rarely induced. When the ERP was shorter (eg, 75 ms for the example shown for placebo in B), prolonged AF was readily induced by a single S₂ in placebo dogs. In mibefradil dogs, even at sites with shorter ERPs (see example B for mibefradil with an ERP of 65 ms), S₂S induced short runs of atrial arrhythmia, which never degenerated into sustained AF. To evaluate inducibility of AF lasting ≥1 second among placebo- versus mibefradil-treated dogs at sites with comparable ERP values, we analyzed the inducibility of AF at all sites with ERP values between 65 and 75 ms, a range for which a significant number of observations could be made for both control and mibefradil dogs. Among placebo-treated dogs, AF was induced with a single extrastimulus at 15 of 26 such sites (58%), which had a mean ERP of 70±1 ms. In contrast, among mibefradil-treated dogs, AF was induced with a single extrastimulus at only 8 of 21 sites (38%, P<0.05 versus placebo) with a mean ERP of 71±1 ms (P=NS versus placebo). Thus, even at sites with matched ERP values, placebo-treated dogs were more vulnerable to AF induction.

Regional Changes in Electrophysiological Properties

The observation of greater atrial vulnerability in placebo versus mibefradil-treated dogs even at sites matched for ERP is compatible with previous observations suggesting that in addition to ERP at the site of stimulation, ERP heterogeneity is an important determinant of AF inducibility with single extrastimuli.¹³ Figure 5 shows an analysis of ERP heterogeneity in 4 different regions in placebo and mibefradil dogs. Compatible with previous observations of regional heterogeneity of tachycardia-induced remodeling, ERP heterogeneity was regionally variable in placebo (but not mibefradil) dogs,
and within-region variability was greater for placebo dogs in 3 of the 4 regions studied.

Plasma Mibefradil Concentrations and Relationship to Electrophysiological Variables
Mean plasma mibefradil concentrations on the day of electrophysiological study averaged 175±47 ng/mL. Figure 6 shows analyses of mean ERP, atrial vulnerability, and AF duration in relationship to plasma drug concentrations in each of the 8 mibefradil-treated dogs. A statistically significant positive correlation was found between ERP and drug concentration (r=0.73, P<0.05), consistent with concentration-dependent drug actions to prevent ERP shortening by atrial tachycardia-induced remodeling. Atrial vulnerability (r=−0.71, P=0.05) and AF duration (r=−0.70, P=0.05) were negatively correlated with drug concentration, compatible with concentration-dependent protection against the AF-promoting effects of rapid pacing.

Effects of Acute Mibefradil Administration
Although all of the observed effects of mibefradil are compatible with a prevention of the effects of tachycardia-induced remodeling, direct electrophysiological effects of the drug are an alternative hypothesis. To evaluate this possibility, we administered mibefradil acutely (25 mg IV) to 5 rapidly paced placebo dogs. Mibefradil did not change mean ERP (75±9 ms versus 86±8 ms after drug, P=NS) or AF cycle length (102±4 versus 105±6 ms, P=NS). In 3 such dogs, mibefradil was administered during AF and did not alter the arrhythmia. To exclude possible contaminating effects of autonomic reflexes in response to acute intravenous mibefradil, the drug was given as a 25-mg IV dose to 5 additional control dogs autonomically blocked with nadolol (0.5 mg/kg IV) and atropine (1 mg/kg IV). Once again, mibefradil did not alter mean ERP (138±4 versus 138±5 ms, P=NS). Plasma concentrations were measured at the time of ERP measurement after intravenous mibefradil and averaged 376±71 ng/mL, higher than the concentrations at the time of electrophysiological study in chronically treated dogs and excluding inadequate plasma concentrations as an explanation for the lack of effects of acute mibefradil administration on ERP or AF. These observations argue strongly against a direct electrophysiological effect of mibefradil as a mechanism for the actions of long-term mibefradil therapy on dogs subjected to rapid atrial pacing and support the notion of a protective effect against tachycardia-induced remodeling.

Discussion
We have found that long-term therapy with mibefradil, a drug with strong T-type Ca\(^{2+}\) channel blocking properties, is highly effective in preventing the induction and maintenance of AF in dogs subjected to 7 days of rapid atrial activation. These effects were related to drug concentration and could not be attributed to direct electrophysiological actions of the drug. The nature of the electrophysiological differences between mibefradil- and placebo-treated dogs is compatible with a prevention of tachycardia-induced electrical remodeling.

Comparison With Previous Studies of Atrial Tachycardia-Induced Electrical Remodeling and Potential Mechanisms
As in previous studies,\(^6\)\(^–\)\(^10\),\(^12\),\(^13\) we found that rapid atrial activation reduces the atrial ERP and wavelength and increases ERP heterogeneity, vulnerability to AF induction by premature beats, and AF duration. We were unable to identify
studies in the literature of the effects of drug intervention during atrial tachycardia on the AF-promoting effects of atrial tachycardias maintained for $>$24 hours. Tieleman et al. showed that verapamil administered to goats during 24 hours of rapid atrial pacing greatly reduces ERP changes caused by atrial tachycardia but has only small effects on the promotion of AF inducibility. During shorter-term AF, verapamil attenuates ERP,$^{10,15,16}$ contractility,$^{21}$ and AF inducibility$^{15,16}$ changes resulting from atrial tachycardia. The ability of mibefradil to prevent electrophysiological changes and AF promotion by 7 days of atrial tachycardia in the present study was striking.

Mibefradil is highly selective for T-type over L-type Ca$^{2+}$ channels (10- to 30-fold selectivity).$^{20}$ The T-type Ca$^{2+}$ channel is not present in all cardiac tissues but appears to be significant in sinoatrial node, Purkinje, and atrial cells.$^{17,20}$ It is inactivated at more negative potentials than the L-type channel,$^{20}$ and T-type current amplitude is quantitatively smaller than L-type current in normal atrial tissue.$^{17}$ Tachycardia-induced atrial remodeling downregulates L-type current without reducing T-type current,$^{17}$ so T-type current may occasion a continuing “spill” of Ca$^{2+}$ into atrial cells undergoing high-frequency activation. Atrial tissue from goats with sustained AF has ultrastructural changes remem
ling those of ventricular myocytes from chronically hibernating myocardium. Thus, anti-ischemic effects related to the T-type Ca\(^{2+}\) channel blocking action of mibefradil may conceivably have contributed to its efficacy in the present study. In addition, T-type current appears to play a particularly important role in hypertrophied or remodeled cardiovascular tissue. It is therefore appealing to speculate that T-type Ca\(^{2+}\) channels may play an important role in atrial tachycardia-induced remodeling; however, because all drugs have potential collateral actions, more evidence is necessary before direct involvement of T-type Ca\(^{2+}\) channels in atrial remodeling can be considered to be established. It is not impossible that mibefradil prevented tachycardia-induced remodeling by another, presently unrecognized, action of the drug. The molecular nature of the T-type current has recently been elucidated by the cloning of a highly mibefradil-sensitive channel with the biophysical properties of I_{CaT} from human heart. The availability of molecular probes may help to clarify the role of T-type channels in electrophysiological remodeling.

**Considerations of the Model**

We used AV block and ventricular pacing to prevent differences in ventricular response rate between placebo- and mibefradil-treated dogs that could affect atrial remodeling independently of any effects on the atria per se. Mibefradil was started 4 days before the beginning of atrial tachycardia because of the long half-life of the drug, which necessitates 3 to 4 days before steady-state tissue levels are achieved. Our findings would therefore be analogous to the long-term use of a drug to prevent remodeling should AF develop, rather than to acute administration after AF begins. Further work to evaluate the efficacy of mibefradil after the onset of atrial tachycardia, as well as its potential ability to reverse remodeling that has already developed, would be interesting but is beyond the scope of the present article. The mean trough plasma concentration of mibefradil in our dogs, 175 ng/mL, is in the range of the plasma concentration produced by standard clinical doses of 50 to 100 mg/d in humans.

The need to use parallel groups of dogs for this type of study is a limitation; because each dog is not its own control, there is an underlying assumption of comparability between groups. To minimize the chances that intergroup differences produce artificial differences, we matched the groups on the basis of animal weight and blinded the drug administration so that subconscious bias did not affect the outcome.

**Novel Aspects and Potential Clinical Relevance**

The present study is, to the best of our knowledge, the first to assess the effects of a pharmacological intervention on tachycardia-induced atrial remodeling over a period >24 hours. Furthermore, the marked attenuation of remodeling-induced changes by mibefradil demonstrates that the prevention of tachycardia-induced atrial remodeling is a feasible goal. Given the great importance of tachycardia-induced remodeling for clinical AF, the possibility that pharmacological therapy can prevent remodeling is encouraging. It lends support to the idea of devising novel therapies that target the development of the substrate for AF, as opposed to traditional antiarrhythmia drug therapy that aims to modify the final electrical product. Mibefradil was recently introduced to the clinical market as an antihypertensive agent; however, because of its propensity to cause serious adverse drug interactions via potent cytochrome P450 inhibition, it has been withdrawn. The direct clinical application of the present findings will therefore have to await the development of I_{CaT} blockers devoid of effects on cytochromes. In the interim, our results are important in showing the feasibility of preventing tachycardia-induced atrial remodeling and by pointing to the potential role of T-type Ca\(^{2+}\) channels in this important phenomenon.

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