The T-Type Ca\(^{2+}\) 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\(^{2+}\) overload is believed to play a role in tachycardia-induced atrial electrophysiological remodeling. L-type Ca\(^{2+}\) 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\(^{2+}\) 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\(^{2+}\) 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
remodeling caused by sustained atrial tachycardia. We therefore compared the effects of mibefradil, a selective ICa blocker,\textsuperscript{18–20} 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 α-chloralose (120 mg/kg IV load, 29.3 mg·kg\textsuperscript{−1}·h\textsuperscript{−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.\textsuperscript{12,13} A mapping system was connected to 5 arrays covering the atrial epicardial surfaces with 240 bipolar electrodes (Figure 3) as previously described.\textsuperscript{13} ERP and conduction velocity (CV) were measured during stimulation at sites in various atrial regions as in previous work.\textsuperscript{13} 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 S2S1 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 S2's 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 S2's 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,\textsuperscript{12} 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-
Overview of the document: The study assesses the effects of a T-type calcium channel blocker on atrial remodeling and atrial fibrillation (AF) in dogs. The research involves pacing and pharmacological interventions to compare the outcomes between control, placebo, and mibefradil-treated groups.

**Study design**

- **Day 1 to Day 12:** This period includes the preparation and treatment phases before rapid atrial pacing begins.
- **Blinded administration of oral placebo or mibefradil-100 mg (1 tablet/day):** This is an important control measure to ensure the study's validity.

**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. Consistent with previous observations, 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.

### 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. 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 before versus 76±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, we found that rapid atrial activation reduces the atrial ERP and wavelength and increases ER 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.\textsuperscript{16} 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,\textsuperscript{10,15,16} contractility,\textsuperscript{21} and AF inducibility\textsuperscript{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\textsuperscript{2+} channels (10- to 30-fold selectivity).\textsuperscript{20} The T-type Ca\textsuperscript{2+} channel is not present in all cardiac tissues but appears to be significant in sinoatrial node, Purkinje, and atrial cells.\textsuperscript{17,20} It is inactivated at more negative potentials than the L-type channel,\textsuperscript{20} and T-type current amplitude is quantitatively smaller than L-type current in normal atrial tissue.\textsuperscript{17} Tachycardia-induced atrial remodeling downregulates L-type current without reducing T-type current,\textsuperscript{17} so T-type current may occasion a continuing “spill” of Ca\textsuperscript{2+} into atrial cells undergoing high-frequency activation. Atrial tissue from goats with sustained AF has ultrastructural changes resem-
blowing those of ventricular myocytes from chronically hibernating myocardium. Thus, anti-ischemic effects related to the T-type Ca\(^{2+}\) channel blocking action of mibe

![Figure 6](Image)

Figure 6. Atrial ERP, atrial vulnerability (% of sites at which AF could be induced), and mean AF duration as a function of mibe

Considerations of the Model

We used AV block and ventricular pacing to prevent differences in ventricular response rate between placebo- and mibe

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 mibe

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