

## Differential efficacy of L- and T-type calcium channel blockers in preventing tachycardia-induced atrial remodeling in dogs

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### Abstract

**Background:** Tachycardia-induced remodeling likely plays an important role in atrial fibrillation (AF) maintenance and recurrence after cardioversion, and  $\text{Ca}^{2+}$  overload may be an important mediator. This study was designed to evaluate the relative efficacies of selective T-type (mibefradil) and L-type (diltiazem)  $\text{Ca}^{2+}$ -channel blockers in preventing tachycardia-induced atrial remodeling. **Methods:** Dogs were given daily doses of mibefradil (100 mg), diltiazem (240 mg) or placebo in a blinded fashion, beginning 4 days before and continuing through a 7-day period of atrial pacing at 400 bpm. An electrophysiological study was then performed to assess changes in refractoriness, refractoriness heterogeneity and AF duration. **Results:** Mean duration of burst-pacing induced AF was similar in placebo ( $567 \pm 203$  s) and diltiazem-treated ( $963 \pm 280$  s,  $P = \text{NS}$ ) animals, but was much less in mibefradil-treated dogs ( $3.6 \pm 0.9$  s,  $P < 0.002$ ) and non-paced controls ( $6.6 \pm 2.7$  s). In contrast to mibefradil, diltiazem did not alter tachycardia-induced refractoriness abbreviation or heterogeneity. To exclude inadequate dosing as an explanation for diltiazem's inefficacy, we studied an additional group of dogs treated with 720 mg/day of diltiazem, and again noted no protective effect. Acute intravenous administration of diltiazem to control dogs failed to alter atrial refractoriness or AF duration, excluding a masking of remodeling suppression by offsetting profibrillatory effects of the drug. **Conclusions:** Whereas the selective T-type  $\text{Ca}^{2+}$ -channel blocker mibefradil protects against atrial remodeling caused by 7-day atrial tachycardia, the selective L-type blocker diltiazem is without effect. These findings are potentially important for understanding the mechanisms and prevention of clinically-relevant atrial-tachycardia-induced remodeling. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Antiarrhythmic agents; Arrhythmia (mechanisms); Ca-channel; Supraventr. arrhythmia

### 1. Introduction

Atrial fibrillation (AF) is an extremely common arrhythmia, with an incidence that is increasing with the aging of the population [1,2]. The treatment of AF remains suboptimal, with antiarrhythmic drugs to maintain sinus rhythm being plagued by a significant risk of ventricular proarrhythmia [3]. Because of the limitations of presently

available therapy, there has been interest in developing drug therapy directed against novel therapeutic targets, including the substrate for arrhythmia [4]. One potentially interesting target is the remodeling process leading to AF-promoting electrophysiological changes that result from atrial tachycardias, particularly AF [5]. Sustained atrial tachycardia causes a variety of electrophysiological alterations, including atrial effective refractory period (ERP) abbreviation [6–10] and increases in refractoriness heterogeneity [10,11], that promote AF inducibility and maintenance.

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There is evidence for  $\text{Ca}^{2+}$ -overload as an initiating signal for tachycardia-induced remodeling [5,9,12]. Experimental and clinical studies suggest that  $\text{Ca}^{2+}$ -channel blockers may prevent the effects of atrial tachycardia on atrial ERP [9,13–15] and mechanical properties [16,17]. The clearest evidence comes from studies of short-term AF, the effects of which on atrial electrophysiology, arrhythmia promotion and mechanical dysfunction are prevented [13,14,16,17]. Studies of longer-term verapamil administration have provided varying results [15,18].

A retrospective analysis of clinical data showed that patients treated with either  $\beta$ -blockers or  $\text{Ca}^{2+}$  antagonists, when considered as a single group, appeared to have reduced AF recurrence after cardioversion (differences were not significant for either the  $\beta$ -blocker or  $\text{Ca}^{2+}$  antagonist group alone) [19]. These findings have led to speculation that  $\text{I}_{\text{Ca,L}}$  blockers may prevent tachycardia-induced remodeling and constitute useful adjunctive therapy for AF patients. This suggestion contrasts with a recent report of apparent inefficacy of verapamil in preventing remodeling caused by long-term atrial tachycardia [18].

In a previous study, we showed that the T-type  $\text{Ca}^{2+}$ -current ( $\text{I}_{\text{Ca,T}}$ ) blocker mibefradil reduces electrophysiological remodeling caused by 7 days of atrial tachycardia in dogs [20]. Although most studies of mibefradil show it to be much more potent as a T-type  $\text{Ca}^{2+}$ -channel blocker than as an L-type blocker [21–23], the drug nevertheless can clearly inhibit L-type channels considerably [24]. The possibility cannot therefore be excluded that mibefradil's  $\text{I}_{\text{Ca,L}}$ -blocking properties are responsible for its ability to prevent atrial tachycardia-induced electrical remodeling. The recently-reported inefficacy of verapamil [18] could have been due to collateral properties, such as verapamil's strong  $\text{K}^+$ -channel blocking action [25], to inadequate oral doses (plasma concentrations were not measured) or to contaminating effects of acute intravenous verapamil administration, which was given to all dogs in the chronic verapamil group at the time of electrophysiological study and could have had acute AF-promoting effects. In order to address the issue of the potential role of T- vs. L-type  $\text{Ca}^{2+}$ -channel blocking action in preventing atrial remodeling, we compared the effects of mibefradil with those of the highly-selective  $\text{I}_{\text{Ca,L}}$ -blocker diltiazem on atrial remodeling caused by 7 days of rapid atrial pacing in the dog. We chose to study diltiazem because, in contrast to verapamil, which inhibits currents carried by the rapid delayed-rectifier  $\text{K}^+$ -clone HERG at similar concentrations to those required to block  $\text{I}_{\text{Ca,L}}$ , diltiazem block of HERG requires concentrations 100-fold greater than those that inhibit  $\text{I}_{\text{Ca,L}}$  [25]. A 7-day period of rapid pacing was chosen because  $\text{I}_{\text{Ca,L}}$  downregulation is quite significant after 7 days, with only small additional decreases noted after 42 days of rapid pacing [26]. Preliminary results of the present study have been presented in abstract form [27].

## 2. Methods

### 2.1. Instrumentation for rapid pacing

Adult mongrel dogs ( $27.1 \pm 0.6$  kg,  $n=37$ ) were anesthetized with pentobarbital (30 mg/kg i.v., additional doses of 4 mg/kg as needed). In an initial procedure, tined unipolar pacing leads were inserted in the right atrial (RA) appendage (RAA) and in the right ventricular (RV) apex under fluoroscopic guidance and connected to pacemakers implanted subcutaneously in the neck. AV block was created with radiofrequency energy (30–40 W for 20–30 s). The average number of radiofrequency applications was  $1.9 \pm 0.4$ . No dogs recovered AV-nodal conduction during the study. The ventricular demand pacemaker was programmed to capture the ventricles at 80 bpm, and the atrial pacemaker was activated to pace the atria with twice-threshold pulses at 400 bpm. Atrial and ventricular pacing were applied continuously during the 7-day rapid atrial pacing period (days 5–12, Fig. 1) prior to electrophysiological study. Animal handling procedures were approved by the Montreal Heart Institute Animal Ethics Committee and followed the guidelines of the Canadian Council for Animal Care (CCAC).

### 2.2. Experimental protocol

In rapidly-paced dogs, one 100 mg tablet/day of mibefradil ( $n=12$  dogs), one 240 mg tablet/day of Cardizem-CD, a slow-release diltiazem preparation ( $n=10$  dogs), or matching placebo ( $n=15$  dogs) was given in a blinded fashion, beginning 4 days before pacemaker implantation and continuing until 24 h before the day of electrophysiological study (Fig. 1). Although we have reported on the effects of mibefradil in this model in a previous publication [20], we felt that it was essential to study the drug in another series of dogs to compare directly and in a blinded fashion the effects of mibefradil with those of diltiazem to minimize the possibility of erroneous conclusions due to time-related and inter-animal variation or to bias. Blood samples were obtained prior to anesthesia on study days for subsequent measurement of plasma diltiazem concentration by high-pressure liquid chromatography. A group of size-matched dogs ( $n=12$ ) without pacemaker implantation was used as a control group (Table 1).

The surface ECG was recorded every 2 days following pacemaker-implantation to confirm maintained atrial and ventricular pacing and AV-block. On the study day, dogs were anesthetized with morphine (2 mg/kg s.c.) and  $\alpha$ -chloralose (120 mg/kg i.v. load, 29.3 mg/kg/h). The atrial pacemaker was then deactivated and a median sternotomy performed. The study preparation and instrumentation were as previously described [10,11]. A mapping system was connected to five arrays covering the atrial epicardial surfaces with 240 bipolar electrodes as in our previous



10 ms longer than the ERP at each site used for ERP determination. The vulnerability at each site was defined by the ability of single  $S_2$ s to induce in a reproducible fashion AF that lasted  $>1$  s. Overall vulnerability in each dog was defined as the percentage of sites at which AF was inducible. Because in some dogs AF was not inducible by single extrastimuli, AF was also induced by stimulating the RAA with 10-Hz, 2-ms stimuli at four times threshold current for 2–10 s. To calculate mean AF duration, AF was induced with burst pacing ten times for AF duration  $<10$  min and twice for AF duration  $>10$  min. AF that lasted  $>20$  min was terminated by direct current electrical cardioversion, and 20 min allowed before repeating AF induction.

#### 2.4. Data analysis

The CV was determined in each region as previously described [11] and the overall CV for each dog calculated from the average of the five regional CV values. Overall wavelength was calculated as the mean of all ERP values in each dog times the mean CV (not significantly different from the value obtained by multiplying ERP in each region by local CV and averaging the values). The coefficient of ERP variance, calculated as  $(S.D./mean) \times 100\%$ , was used as an index of ERP heterogeneity. The number of sites for ERP determination in each region was equivalent across dogs and between groups.

Statistical comparisons between two groups only were performed by Student's *t*-test or the Mann–Whitney rank sum test (for non-normally distributed data). Analysis of variance (ANOVA, for parametric data) or a Kruskal–

Wallis rank sum test (when data could not be assumed to be normally-distributed) was used for multiple-group comparisons, followed by a range test. A chi-square test was used for contingency comparisons. Averages are given as mean  $\pm$  S.E.M., and a two-tailed  $P < 0.05$  was considered statistically-significant.

### 3. Results

#### 3.1. Overall electrophysiological changes

Control, placebo, mibefradil and diltiazem dogs showed no significant differences in size, number of sites for ERP determination, and atrial diastolic threshold (Table 1). Mibefradil-treated dogs had a slower sinus rate compared to placebo, control and diltiazem dogs, consistent with the role of T-type  $Ca^{2+}$  channels in sinus-node automaticity [21].

#### 3.2. Effects on AF vulnerability and maintenance

Placebo-treated dogs subjected to 7-day rapid atrial pacing had significantly-increased AF duration (Fig. 2A) and vulnerability to AF induction (Fig. 2B). AF duration of mibefradil-treated dogs ( $6.6 \pm 2.7$  s) was reduced compared to placebo-treated dogs ( $567 \pm 203$  s,  $P < 0.002$ ) and similar to non-paced control dogs ( $3.6 \pm 0.9$  s,  $P = NS$ ). In contrast, the AF duration of diltiazem-treated rapidly-paced dogs ( $963 \pm 280$  s) was not significantly different from placebo-treated rapidly-paced dogs and substantially greater than non-paced controls ( $P < 0.002$ ). Changes in

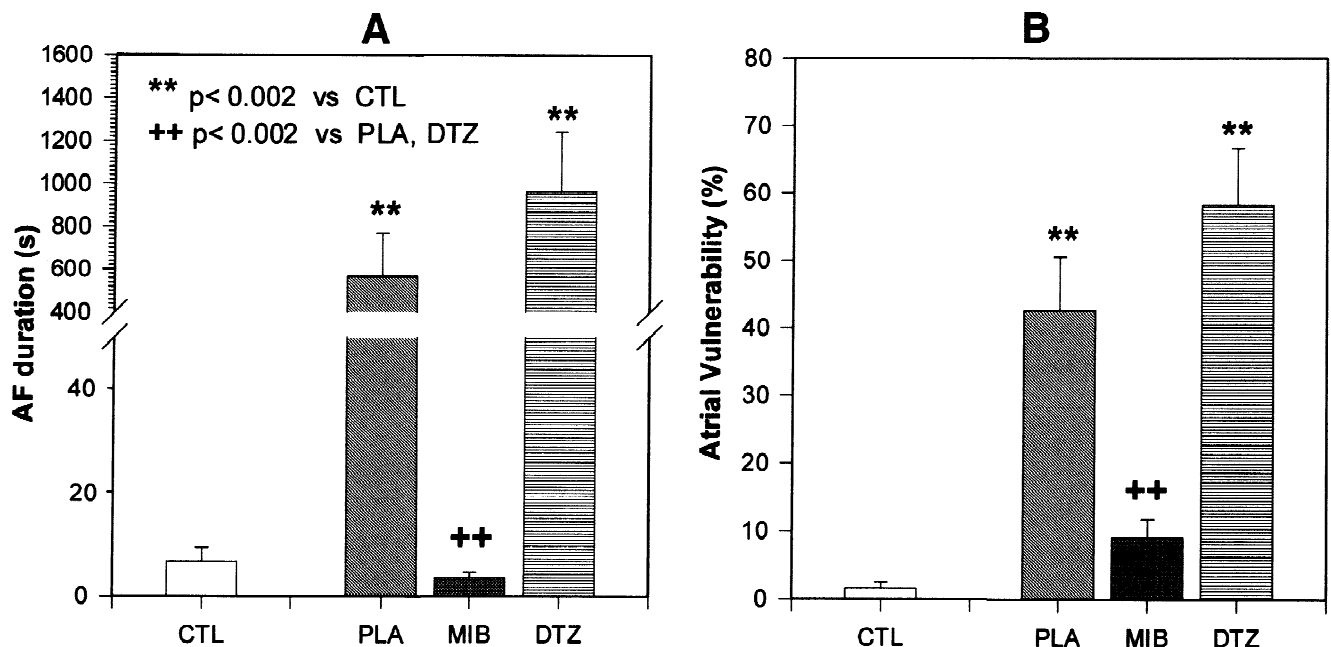


Fig. 2. Changes in AF duration (A) and AF vulnerability (B) induced by 7 days of rapid atrial pacing in placebo (PLA), mibefradil (MIB) and diltiazem (DTZ) dogs. \*\*  $P < 0.002$  vs. CTL, ++  $P < 0.002$  vs. PLA and DTZ.

vulnerability to AF induction by single atrial premature complexes were qualitatively similar to those in AF duration. In placebo-treated dogs, rapid pacing led to a highly-significant increase in vulnerability, with AF induced by single extrastimuli at over 40% of sites per dog, compared to about 2% in non-paced controls ( $P < 0.002$ , Fig. 2B). Mibefradil greatly decreased vulnerability (AF induced at fewer than 10% of sites,  $P < 0.002$  vs. placebo). Diltiazem administration was no better than placebo in reducing AF vulnerability in rapidly-paced dogs, with AF inducible at nearly 60% of sites ( $P = \text{NS}$  vs. placebo,  $P < 0.002$  vs. mibefradil).

### 3.3. Changes in electrophysiological properties

Placebo-treated dogs subjected to 7 days of rapid-pacing had significantly reduced ERP (Fig. 3A) and unchanged CV (Fig. 3B). Wavelength changes paralleled those in ERP (Fig. 3C). In addition to decreasing the absolute ERP value, rapid pacing also increased ERP heterogeneity (Fig. 3D). Mibefradil prevented the ERP-abbreviating effect of rapid pacing, with the mean ERP in mibefradil-treated dogs ( $103 \pm 5$  ms) not significantly different from the

average value in non-paced control dogs ( $114 \pm 3$  ms). In diltiazem-treated rapidly-paced dogs, ERP averaged  $74 \pm 4$  ms, similar to placebo-treated animals ( $79 \pm 3$  ms), with both significantly reduced from control non-paced dogs (Fig. 3A). Wavelength changes (Fig. 3C) reflected ERP alterations, with mibefradil showing significant protection against tachycardia-induced abbreviation and diltiazem no effect. In contrast to placebo-treated rapid-pacing dogs, mibefradil-treated dogs had a coefficient of variation in ERP ( $17.0 \pm 1.2\%$ ) which was not significantly different from the value in control dogs ( $14.1 \pm 0.8\%$ ). Diltiazem-treated rapidly-paced dogs had similar ERP heterogeneity ( $28.3 \pm 2.0\%$ ) to placebo animals ( $26.7 \pm 1.9\%$ ), with both values significantly greater than in control (non-paced) and mibefradil-treated rapidly-paced dogs.

### 3.4. Regional changes in electrophysiological properties

Regional ERP differences under control conditions in dogs are related to discrete ionic current distributions [28], and increased ERP heterogeneity is important in tachycardia-induced remodeling [11]. Fig. 4 shows an analysis of changes produced by each intervention in ERP values

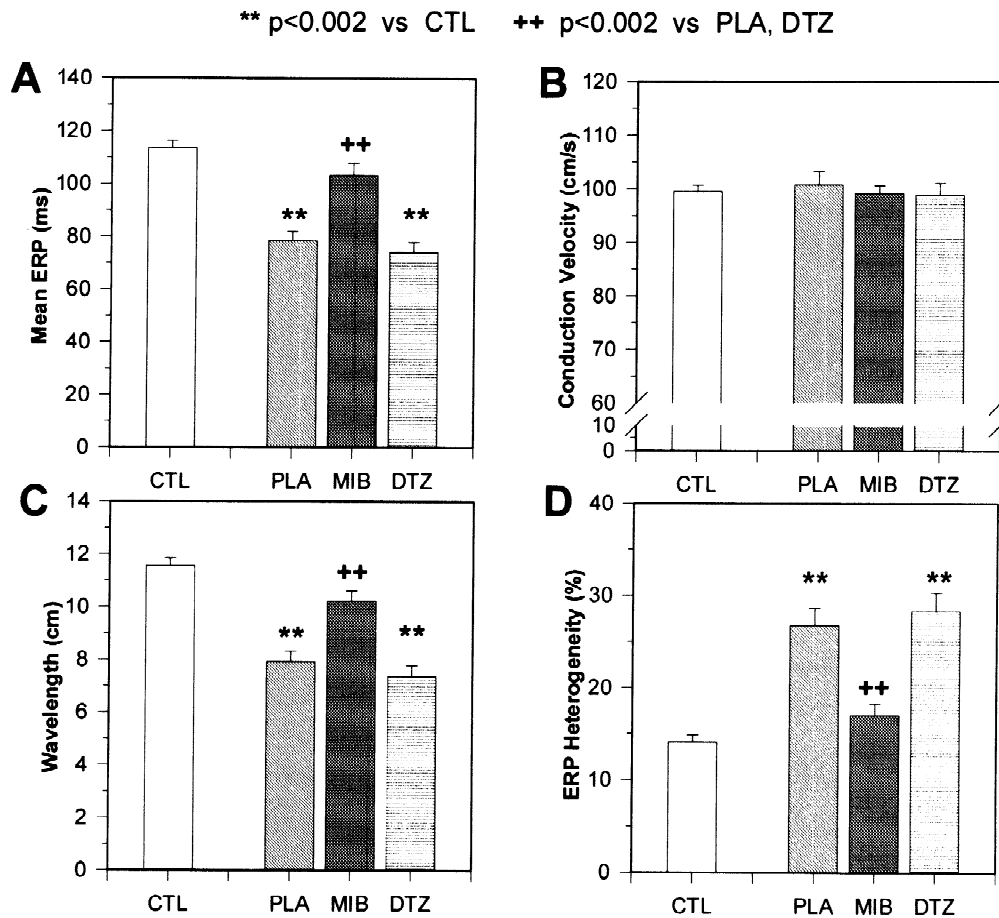


Fig. 3. Changes in ERP (A), conduction velocity (B), wavelength (ERP $\times$ CV) (C) and ERP heterogeneity (D) at a BCL of 300 ms induced by 7 days of rapid atrial pacing in placebo (PLA), mibefradil (MIB) and diltiazem (DTZ) groups. \*\*  $P < 0.002$  vs. CTL, ++  $P < 0.002$  vs. PLA and DTZ.

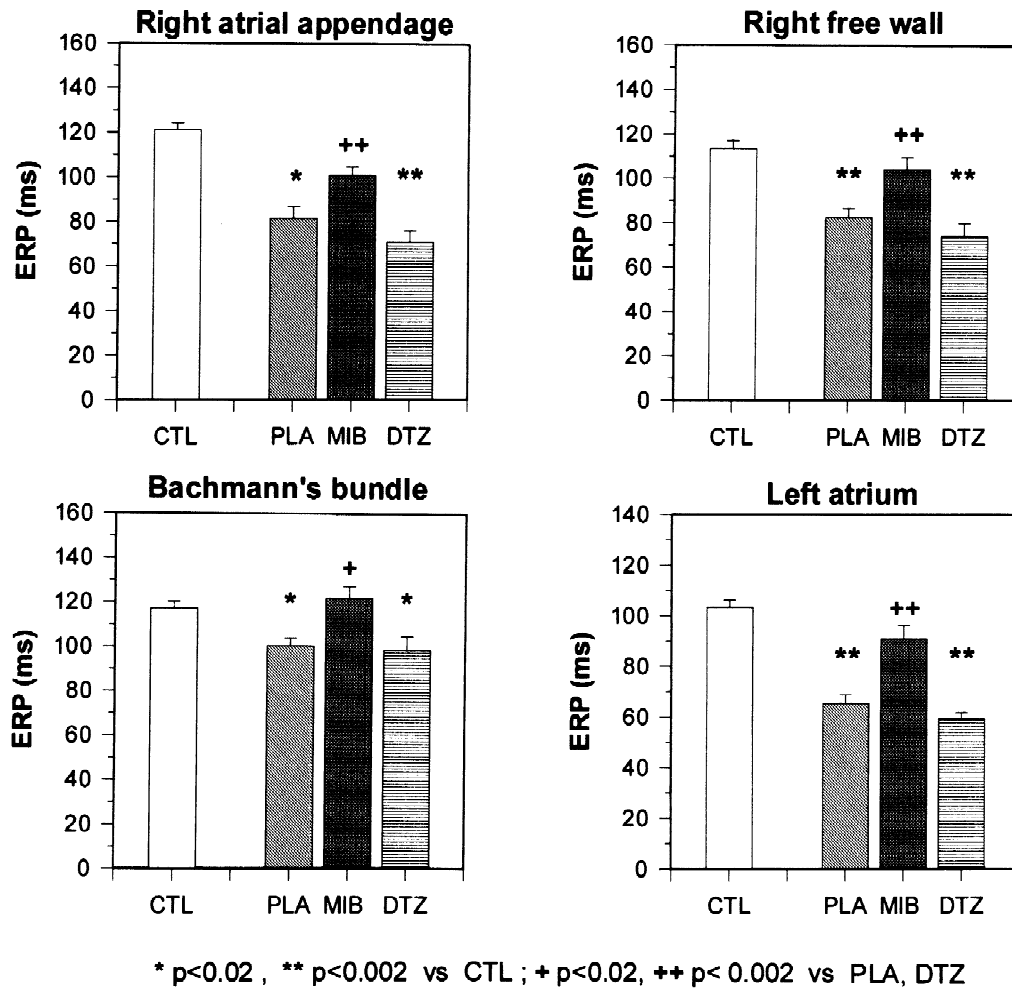


Fig. 4. Changes in intraregional atrial ERP induced by rapid atrial pacing in placebo (PLA), mibefradil (MIB) and diltiazem (DTZ) dogs. \*  $P < 0.02$ , \*\*  $P < 0.002$  vs. CTL, +  $P < 0.02$ , ++  $P < 0.002$  vs. PLA and DTZ.

for each of four regions for which sufficient data were available. The results indicate that for each region studied, rapid pacing significantly decreased ERP values in placebo and diltiazem-treated dogs, but had much less (and statistically non-significant) effect in mibefradil-treated animals.

### 3.5. Diltiazem plasma concentrations and effects of high-dose diltiazem

In our previous study, we showed that plasma mibefradil concentrations were significantly related to AF duration and ERP in individual rapidly-paced dogs, consistent with concentration-dependent protection [20]. The effective trough mibefradil concentrations in that study were in the range of 100–400 ng/ml (0.2–0.7  $\mu\text{M}$ ). We were unable to obtain mibefradil plasma concentration measurements for the dogs in the present study. However, we did measure plasma diltiazem concentrations and evaluated the relationship with AF duration or atrial ERP. The correlation coefficients were  $-0.42$  (for AF duration) and  $-0.12$  for

ERP ( $P = \text{NS}$  for each), consistent with diltiazem's lack of effect on mean ERP and AF duration.

The lowest diltiazem concentration 24 h after the last dose of diltiazem averaged  $28 \pm 7$  ng/ml, just below the therapeutic concentration of 40 ng/ml [29]. We therefore considered the possibility that diltiazem's inefficacy was due to insufficient plasma concentrations and studied an additional group of four dogs treated with 3-fold larger doses, 720 mg/day (on a per-weight basis, equivalent to about 1680 mg/day, or five times the maximum clinical dose, in man). The same protocol as shown in Fig. 1 was used, except that the dose of diltiazem consisted of four 180-mg tablets each morning. This dose significantly reduced arterial pressure, from  $122 \pm 8/66 \pm 5$  mmHg in placebo dogs to  $99 \pm 4/44 \pm 3$  mmHg in diltiazem-treated dogs ( $P = 0.01$ ). The sinus cycle length was also significantly prolonged by high-dose diltiazem, from  $396 \pm 28$  to  $605 \pm 38$  ms ( $P < 0.05$ ), indicating significant pharmacological effects. After 7 days of rapid pacing, ERP in these dogs (BCL 300 ms) was  $81 \pm 10$  ms, not significantly different from placebo-treated dogs subjected to atrial

tachycardia. Similarly, AF duration averaged  $1342 \pm 428$  s (vs.  $567 \pm 203$  s for placebo-treated rapidly-paced dogs), AF was inducible at  $63 \pm 24\%$  of sites (compared to  $43 \pm 8\%$  for placebo), and the coefficient of ERP heterogeneity averaged  $24 \pm 3\%$  (vs.  $27 \pm 2\%$  for placebo), none significantly different from atrial-tachycardia dogs treated with placebo or low-dose diltiazem. Thus, high-dose diltiazem had no greater anti-remodeling effect than low-dose and the lack of diltiazem effect cannot be attributed to inadequate dosing.

### 3.6. Effects of acute diltiazem administration

The acute administration of  $\text{Ca}^{2+}$  antagonists has been reported to promote AF maintenance [30]. We considered the possibility that the lack of benefit from diltiazem may have been due to an acute pro-fibrillatory effect of the drug, i.e. that remodeling might have been prevented, but that residual drug present in the blood may have itself decreased ERP and promoted AF, offsetting the effects mediated by inhibition of remodeling. To evaluate this possibility, we infused diltiazem intravenously to four additional non-paced dogs at loading and maintenance doses previously shown to produce stable plasma diltiazem concentrations [31]. Acute administration of diltiazem did not significantly alter atrial ERP, which averaged  $112 \pm 2$  ms (BCL 300 ms) before and  $116 \pm 3$  ms after the drug, nor AF duration, which averaged  $8.4 \pm 6.0$  and  $25.8 \pm 14.6$  s, respectively.

## 4. Discussion

We have shown that the  $\text{Ca}^{2+}$ -channel blockers diltiazem and mibefradil, with different selectivities for T- vs. L-type channels, have markedly different effects on electrophysiological remodeling caused by 7-day atrial tachycardia. Whereas the T-type blocker mibefradil strongly inhibits the ERP-altering and AF-promoting effects of atrial tachycardia, the L-type blocker diltiazem has no significant effects.

### 4.1. Relationship to previous studies of $\text{Ca}^{2+}$ channel blockers in tachycardia-induced remodeling

L-type  $\text{Ca}^{2+}$  channel blockade has been shown to inhibit atrial remodeling caused by short-term (10–20 min) atrial tachycardia [13,14,16,17]. ERP changes induced by 7–24 h of atrial tachycardia are attenuated by verapamil [8,15,18]. Verapamil appears to have no effect on ERP or AF vulnerability changes induced by longer-term atrial tachycardia [18], although in the latter study plasma drug concentrations were not measured to ensure that adequate blood levels were achieved by oral dosing. We previously showed that mibefradil attenuates tachycardia-induced ERP, AF duration and AF vulnerability changes [20]. In

the present study, we compared directly the effects of mibefradil with those of the  $\text{I}_{\text{Ca,L}}$  blocker diltiazem, finding that the latter does not share mibefradil's protective actions. The discrepancy between our finding of inefficacy of  $\text{I}_{\text{Ca,L}}$  blockade in 7-day atrial tachycardia-induced remodeling and previous studies of  $\text{I}_{\text{Ca,L}}$  blockers in tachycardia-induced remodeling may be due to the duration of atrial tachycardia. Most previous studies involved atrial tachycardia of <24-h duration. The mechanisms of short-term atrial-tachycardia remodeling are likely to be quite different from those of longer-term remodeling, the former being primarily functional and the latter related to altered gene-expression [5]. Thus, the role of  $\text{I}_{\text{Ca,L}}$  may well be different in mediating the effects of shorter-term vs. longer-term tachycardia. In agreement with this idea, the effects of verapamil on AF promotion caused by atrial tachycardia lasting 24 h or greater [15,18] are very different from its clear efficacy against AF promotion after 10–20 min of AF [12,13].

### 4.2. Novel findings and potential significance

This is the first study of which we are aware to compare simultaneously the effects of a selective  $\text{I}_{\text{Ca,L}}$  blocker and a selective  $\text{I}_{\text{Ca,T}}$  blocker on tachycardia-induced remodeling in a controlled fashion. The results clearly showed no benefit from the selective  $\text{I}_{\text{Ca,L}}$  blocker diltiazem, in contrast to the clear efficacy of the  $\text{I}_{\text{Ca,T}}$  blocker mibefradil.  $\text{I}_{\text{Ca,L}}$  down-regulation appears to play a central role in long-term atrial tachycardia-induced action potential duration and refractory period changes [26]. Tachycardia-related ionic alterations are caused by decreased concentrations of mRNA encoding ion channel  $\alpha$ -subunits [32]. The nature of the signal transduction system leading to ion-channel mRNA changes is unknown, but our findings point to a potentially-important role of  $\text{I}_{\text{Ca,T}}$ . The absolute magnitude of  $\text{Ca}^{2+}$  entry via  $\text{I}_{\text{Ca,T}}$  seems to be substantially less than that via  $\text{I}_{\text{Ca,L}}$  for normal atrial myocytes [26]. On the other hand,  $\text{I}_{\text{Ca,L}}$  decreases with tachycardia, as a result of both functional accumulation of inactivation [33] and ion-channel down-regulation [32,34,35]. It is therefore possible that at sustained rapid rates  $\text{I}_{\text{Ca,T}}$  plays a relatively more important role in  $\text{Ca}^{2+}$  entry. The negative findings with diltiazem indicate that  $\text{I}_{\text{Ca,L}}$  inhibition alone is insufficient to prevent tachycardia-induced remodeling; however, they do not exclude the possibility that inhibition of both  $\text{I}_{\text{Ca,L}}$  and  $\text{I}_{\text{Ca,T}}$  is needed, since mibefradil can affect both  $\text{Ca}^{2+}$ -currents [21–24]. It may be that the prevention of  $\text{Ca}^{2+}$  overload during long-term tachycardia (>24 h) requires the inhibition of both  $\text{Ca}^{2+}$  entry pathways. Alternatively,  $\text{I}_{\text{Ca,T}}$  may have privileged access to systems regulating DNA transcription.

Clinically, the notion of preventing atrial tachycardia-induced remodeling is an attractive new approach to improving the management of AF [5,19]. Tielemans et al.

[19] analyzed retrospectively the rate of reversion to AF following cardioversion. They found that patients taking  $Ca^{2+}$ -channel blockers (including verapamil, diltiazem and dihydropyridines, breakdown not specified) had a greater tendency to remain in sinus rhythm ( $P=0.06$ ). Significant ( $P=0.03$ ) improvements in sinus rhythm maintenance were noted when patients on  $\beta$ -blockers were grouped together with patients on  $Ca^{2+}$ -antagonists and considered to constitute an ‘intracellular  $Ca^{2+}$ -lowering drug’ group. Our findings, particularly in combination with the similar observations on verapamil of Lee et al. [18], raise questions about the ability of  $I_{Ca,L}$  blockers to prevent atrial tachycardia-induced electrophysiological remodeling. A recent study [36] showed early recurrence (within 24 h) following electrical cardioversion of AF in two of 44 diltiazem-treated patients compared to three of 30 patients on digoxin ( $P=NS$ ). Recurrence rates at 1 month were also similar in diltiazem (56%) and digoxin (78%) treated patients. The present findings confirm our previous work [20], showing that it is possible to prevent pharmacologically the electrophysiological consequences of atrial tachycardia-related remodeling. Mibefradil has been removed from the market because of adverse drug interactions [37], but further work is certainly warranted to develop more selective T-type  $Ca^{2+}$ -channel blockers without effects on cytochromes, that might be safer and equally efficacious against atrial remodeling. It is intriguing that amiodarone is a potent T-type  $Ca^{2+}$ -channel blocker, with greater effects on T-type than L-type channels at normal diastolic potentials [38]. This observation raises the possibility that amiodarone’s superior efficacy in the treatment of AF [39] may be, at least in part, due to prevention of tachycardia-related remodeling, in addition to its potent  $K^+$  channel blocking properties [40].

#### 4.3. Potential limitations

Any study with parallel populations is open to possible influences of bias and random variation among groups. Bias was minimized by blinded drug administration. Random variation seems unlikely in view of the clear and consistent effects seen.

We chose to study 7 days of tachycardia as the time when ionic and refractory period changes approach steady-state [10,26]. The results might have been different with a longer period of rapid atrial pacing. Dogs were begun on therapy 4 days before atrial-tachycardia initiation to ensure steady-state loading at the onset of tachycardia. Clinically, this would correspond to the response of patients already taking mibefradil or diltiazem when AF begins. Further work will be needed to determine whether these agents can reverse the effects of remodeling if they are begun after tachycardia is established.

Mibefradil was extremely effective in preventing atrial remodeling. The present study shows that  $I_{Ca,L}$  inhibition does not mediate its protective action, but does not prove

that  $I_{Ca,T}$  inhibition is the mechanism of protection. Other possibilities, including  $K^+$ -channel [41] and cytochrome [42] inhibition, as well as presently unrecognized actions of the drug, could also have played a role.

We used radiofrequency ablation to prevent variations in ventricular response rate (due to drug effects on the AV node) from contaminating the results. Control of the ventricular rate was essential, because otherwise there may have been a more rapid ventricular rate in control than drug (especially diltiazem) treated dogs. This could have resulted in drug effects on atrial remodeling due to differences in ventricular function rather than atrial remodeling per se. The potential disadvantages of this approach include atrial consequences of loss of AV synchrony and possible (albeit unlikely) direct effects of AV nodal ablation on the atria. Since any such collateral effects would have applied equally to placebo, mibefradil and diltiazem groups, we don’t believe they affected our results.

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