Effects of Antiarrhythmic Drugs on Fibrillation in the Remodeled Atrium
Insights Into the Mechanism of the Superior Efficacy of Amiodarone

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Background—The basis of the unique effectiveness of amiodarone for atrial fibrillation (AF) is poorly understood. The present study tested the hypothesis that amiodarone blocks electrical remodeling induced by atrial tachycardia.

Methods and Results—Mongrel dogs were subjected to atrial tachycardia (400 bpm for 7 days) in the absence and presence of therapy with amiodarone, the class III cardiac antiarrhythmic drug dofetilide, or the class I agent flecainide begun 3 days before the onset of tachypacing and maintained until a final electrophysiological study. AF vulnerability (percentage of sites with AF induction by single premature extrastimuli), mean AF duration, atrial effective refractory period (ERP), and conduction velocity were compared among these dogs and in unpaced dogs in the absence or presence of treatment with the same agents. Only amiodarone prevented promotion of AF duration and vulnerability by atrial tachycardia. Furthermore, only amiodarone eliminated tachycardia-induced ERP abbreviation and loss of ERP rate adaptation while obviating L-type Ca2+-current α1c-subunit downregulation as determined by Western blot. In an additional series of dogs monitored with repeated electrophysiological studies, amiodarone administered after the induction of atrial tachycardia remodeling reversed remodeling within several days, despite continued atrial tachypacing during amiodarone therapy.

Conclusions—Amiodarone is uniquely effective against AF promotion by atrial tachycardia remodeling in this experimental model and prevents electrophysiological and biochemical consequences of remodeling. Amiodarone also reversed remodeling established by 4 days of atrial tachycardia. The inhibition of atrial tachycardia remodeling may therefore contribute to the superior efficacy of amiodarone in AF. (Circulation. 2003;107:1440-1446.)

Key Words: electrocardiography ■ ion channels ■ arrhythmia

Atrial fibrillation (AF) is the most common cardiac arrhythmia, with age-related prevalence reaching >10% in octogenarians.1 AF treatment is suboptimal at present, and AF is the most common pathogenic factor for stroke in the elderly.2 Recent evidence indicates that disease- or arrhythmia-induced alterations in cardiac electrophysiology (electrical remodeling) are central in arrhythmia genesis,3 particularly for AF, which alters cardiac electrophysiology to promote its own maintenance.4–6 A key component of AF-related remodeling is downregulation of L-type Ca2+-channel protein, which reduces atrial effective refractory period (ERP) and decreases physiological ERP rate adaptation.7,8

Two recent controlled studies indicate that amiodarone is uniquely effective in treating AF9,10; however, the underlying mechanistic basis is unknown. Mibefradil, a T-type Ca2+-current blocker, prevents atrial electrical remodeling11,12 but was never studied in clinical AF and is no longer clinically available because of adverse drug interactions related to CYP 3A4 inhibition. Amiodarone also has T-type Ca2+-channel-blocking properties.13 We therefore speculated that the superior efficacy of amiodarone in AF might be related to inhibition of electrical remodeling. The present study was designed to (1) compare the antiarrhythmic properties of chronically administered amiodarone with a class I (flecainide) and a class III (dofetilide) antiarrhythmic drug in atrial tachycardia–associated AF and (2) determine whether these compounds attenuate the effects of atrial tachycardia on atrial electrophysiology and L-type Ca2+-channel expression. Because our findings suggested that amiodarone prevents the development of atrial tachycardia remodeling, we also assessed whether amiodarone administered after the development of atrial tachycardia remodeling can reverse remodeling despite continued atrial tachycardia.

Animal Model
Fifty-three mongrel dogs (18 to 39 kg) were used. Details of the model have been published previously.11,12,14,15 Dogs were initially anesthetized with ketamine (5.3 mg/kg IV), diazepam (0.25 mg/kg IV), and halothane (1% to 2%). Unipolar pacing leads for ventricular and atrial pacing were inserted fluoroscopically into the right ventricular (RV) apex and the right atrial (RA) appendage. AV block was created by radiofrequency catheter ablation and the right
ventricular pacemaker programmed to capture at 80 bpm. Twenty-four hours later, atrial tachypacing was initiated at 400 bpm and maintained for 7 days (as for studies of drug effects on atrial tachycardia associated AF) or 10 days (as for studies of amiodarone-induced reversal of remodeling). ECGs were verified every other day to ensure 1:1 atrial capture.

**Evaluation of the Ability of Amiodarone to Prevent Remodeling**

Results in 10 tachypaced dogs receiving no drug (A+ group) were compared with results in 6 dogs per group treated with a test drug before and during tachypacing. These results were compared with those of unpaired control dogs (n=10) and of dogs receiving the same doses of drugs without tachypacing (n=5 per group). Drug doses were as follows: amiodarone, 1800 mg/d for 2 days followed by 1200 mg/d for 8 days; dofetilide, 500 μg/d; and flecainide, 200 mg/d. The doses of flecainide and dofetilide were based on clinical maintenance doses, with a 3-day treatment period before tachypacing calculated to produce steady-state conditions. For amiodarone, which reaches steady state over months on maintenance therapy, a loading dose was used to achieve therapeutic effects rapidly, as often used clinically. Each drug therapy group was studied successively. In atrial tachypaced dogs, treatment began 3 days before atrial pacemaker activation and continued until the morning of the electrophysiological study to ensure therapeutic concentrations throughout tachypacing.

After the conditioning period (no intervention, 7 days of tachypacing with or without drug, or 7 days of drug without tachypacing), dogs were anesthetized with morphine (2 mg/kg SC) and ketamine/diazepam/isoflurane anesthesia, and then the atrial tachypacemaker and right ventricular pacemaker were implanted, and AV block was created as described above. A median thoracotomy was performed, and a bipolar Teflon-coated stainless-steel electrode was inserted into the left atrium (LA) for programmed stimulation. A 240-bipolar electrode array was sewn onto the atrial epicardial surfaces, and selected LA and RA sites were used for programmed stimulation. An atrial bipolar lead was implanted for programmed stimulation and recording during electrophysiological study. After 24 hours of recovery, a baseline electrophysiological study was performed under ketamine/diazepam/isoflurane anesthesia, and then the atrial tachypacemaker was programmed to capture the RA at 400 bpm for 10 days. Electrophysiological studies were repeated in the same dogs.

**L-Type Ca²⁺-Channel α₁D-Subunit Expression**

At the end of open-chest studies, atrial tissues were fast-frozen in liquid nitrogen and stored at −80°C. Subsequently, the tissue samples were homogenized in RIPA buffer. The suspension was incubated on ice and centrifuged (14000 g, 10 minutes, 4°C), and the supernatant was stored at −80°C. Protein concentration was determined by Bradford assay, with bovine albumin as a standard, to ensure equal protein loading. Protein extracts (200 μg) were denatured in Laemmli buffer and electrophoresed on 7.5% SDS-polyacrylamide gels. Proteins were transferred to polyvinylidene difluoride membranes, blocked with 5% nonfat dry milk in Tris-buffered saline (TBS), and incubated with primary antibody (Alomone, anti-cardiac α₁D) for 4 hours. After 3 washes in 0.1% Tween 80–TBS (TTBS), membranes were reblocked in 1% nonfat dry milk in TTBS for 10 minutes and then incubated with secondary antibody (Jackson Laboratories, goat anti-rabbit) for 40 minutes, followed by 3 additional washes in TTBS. Antibody detection was performed with Western blot Chemiluminescence Reagent Plus. Band densities were quantified by densitometry (Quantity One software) standardized to average control values.

**Reversal of Atrial Tachycardia–Induced Remodeling**

In 5 dogs, an atrial tachypacemaker and right ventricular pacemaker were implanted, and AV block was created as described above. Atrial bipolar leads were implanted for programmed stimulation and recording during electrophysiological study. After 24 hours of recovery, a baseline electrophysiological study was performed under ketamine/diazepam/isoflurane anesthesia, and then the atrial tachypacemaker was programmed to capture the RA at 400 bpm for 10 days. Electrophysiological studies were repeated in the same dogs.

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**Figure 1.** Effects of study drugs on AF promotion by atrial remodeling. Left, Mean±SEM AF duration in control (CTL) dogs; dogs treated with amiodarone (Amio), dofetilide (Dof) or flecainide (Fle) without tachypacing; tachypaced dogs without drug therapy (A+); and tachypaced dogs receiving drug therapy (Drug name/A+). Right, Atrial vulnerability in same groups as at left. *P<0.05, ††P<0.01, vs CTL; †††P<0.01 vs A+.
under ketamine/diazepam/isoflurane at 2, 4, 7, and 10 days after the beginning of atrial tachypacing, with amiodarone administered from day 5.

Data Analysis

Multiple-group statistical comparisons were obtained by ANOVA. A t test with Bonferroni correction was used to evaluate differences between individual means. Average results are given as mean±SEM, and a 2-tailed value of $P<0.05$ was considered statistically significant.

Results

Remodeling-Related AF Promotion and Electrophysiology

Changes in susceptibility to AF, as determined during the terminal open-chest study after the completion of 7 days of tachypacing, are shown in Figure 1. The bar graphs on the left indicate the mean duration of AF episodes induced by brief bursts of atrial pacing. In dogs not subjected to tachypacing, mean AF duration was very brief. Atrial tachypacing (A+) increased mean AF duration significantly, to >600 seconds. Amiodarone therapy (Figure 1A) completely prevented the increase in AF duration caused by tachypacing (Amio/A+). In contrast, neither dofetilide (Figure 1B) nor flecainide (Figure 1C) significantly altered the AF duration–prolonging effect of atrial tachypacing. The graphs on the right of Figure 1 show effects on another index of tachypacing-induced AF promotion, the enhancement of AF vulnerability. In the absence of atrial tachypacing, atrial vulnerability was relatively low, with AF inducible at <20% of sites. Atrial tachypacing substantially increased atrial vulnerability to AF induction, with AF inducible at >50% of sites. This effect was completely prevented by amiodarone (Figure 1D), but neither dofetilide (Figure 1E) nor flecainide (Figure 1F) prevented significant atrial tachypacing–induced increases in vulnerability. Thus, of the antiarrhythmic drugs tested, only amiodarone prevented AF promotion by atrial tachycardia remodeling.

Figure 2 shows the ERP changes induced by remodeling in the presence and absence of the various drugs studied. In the absence of drug therapy, ERP was strongly reduced by atrial tachypacing, and the normal rate adaptation of ERP was lost (A+, Figure 2, left). Neither dofetilide (Figure 2B) nor flecainide (Figure 2C) significantly altered the ERP changes induced by tachypacing. In contrast to treatment with the other drugs, therapy with amiodarone was associated with a complete prevention of the ERP-shortening effects of atrial
tachypacing (Amio/A+; Figure 2A). Changes in ERP per se may be caused by a direct action of antiarrhythmic drugs on cardiac cell-membrane ion channels or an interaction with remodeling. Conversely, the abolition of ERP rate adaptation is a characteristic effect of atrial tachycardia–induced remodeling. We therefore specifically analyzed the effects of various drug interventions on the degree of ERP rate adaptation between BCLs of 360 and 150 ms, as shown in the right panels of Figure 2. In the absence of tachypacing, none of the drugs significantly affected ERP rate adaptation. Atrial tachypacing abolished rate adaptation, an effect that was not significantly altered by treatment with dofetilide (Figure 2E) or flecainide (Figure 2F). In the presence of amiodarone, however, ERP rate adaptation was not significantly altered by 7-day atrial tachypacing (Figure 2D, Amio/A+).

Figure 3 shows an evaluation of the effects of atrial tachypacing and drug therapy on ERP in 7 atrial regions. Atrial tachypacing alone substantially decreased ERP in all regions, although the extent of ERP reduction varied. In the presence of amiodarone, tachypacing did not reduce ERP to below control drug-free values (Figure 3A). In contrast to amiodarone, neither dofetilide (Figure 3B) nor flecainide (Figure 3C) significantly altered the effect of atrial tachypacing. If amiodarone attenuates atrial remodeling, one would expect its ERP-prolonging action to be greater in the presence of remodeling than in its absence. Figure 4 shows an analysis of the percentage increase in ERP caused by each agent in both the absence (drug alone versus unpaced control) and presence (drug/A+ versus A+ alone) of atrial tachypacing. Amiodarone (Figure 4A) clearly produced larger ERP increases in the presence of tachypacing than in its absence. In contrast, dofetilide produced small and variable ERP increases in both the absence and presence of tachypacing, whereas flecainide had no perceptible effect on ERP.

The enhancement of the effect of amiodarone on ERP in the presence of atrial tachycardia remodeling could be caused by drug-induced antagonism of the remodeling effects on ERP or a generalized amiodarone effect–enhancing action of remodeling. To address this issue, we examined drug effects on CV. Figure 5 shows RA and LA CV in the absence of drug and with tachycardia remodeling and each drug studied. Atrial tachycardia alone for 7 days had no effect on CV, as previously reported.4,15,16 Amiodarone significantly reduced CV in both the RA and LA (Figure 5A), but its effects on CV were the same in the absence and presence of tachycardia remodeling. Dofetilide had no effect on CV (Figure 5B), as expected. Flecainide reduced CV moderately in both the absence and presence of remodeling (Figure 5C). The CV data do not support the notion of a generalized enhancement of the actions of amiodarone by atrial tachycardia.
Changes in $\alpha_{1c}$-Subunit Expression
The results presented above are compatible with the notion that amiodarone inhibits the development of atrial tachycardia remodeling. To address this issue more directly, we quantified protein expression of the L-type $\text{Ca}^{2+}$-channel $\alpha_{1c}$-subunit (Ca $v$ 1.2) with the use of Western blot techniques. A clear signal was obtained at a molecular weight just above 200 kDa, corresponding to the expected molecular weight of Ca $v$ 1.2. Atrial tachypacing alone substantially decreased the intensity of the signal (Figure 6A). Amiodarone did not appreciably alter signal intensity in the absence of tachypacing (Figure 6B) but did prevent the reduction produced by tachypacing. Neither dofetilide (Figure 6C) nor flecainide (Figure 6D) significantly affected Ca $v$ 1.2 signal intensity in the absence of tachypacing, nor did they alter the tachypacing-induced reductions. The contrast between the lack of effect of flecainide and dofetilide on pacing-induced Ca $v$ 1.2 protein downregulation and the protective effect of amiodarone is illustrated by the mean data in Figure 6E.

Reversal of Remodeling
The studies described above show that amiodarone can prevent the development of atrial remodeling. Because antiarrhythmic drugs are often given to patients already in AF, after atrial remodeling has occurred, we examined the ability of amiodarone to reverse already established atrial remodeling. Figure 7A shows the evolution of AF duration and atrial ERP in 5 dogs studied repeatedly with indwelling atrial electrodes during 4 days of atrial tachypacing followed by 6 days of tachypacing during amiodarone therapy. Atrial tachypacing significantly reduced ERP and increased AF duration. The subsequent administration of amiodarone restored ERP, ERP rate adaptation (Figure 7B), and AF duration to their control values, despite concomitant tachypacing.

Discussion
In the present study, amiodarone was found to have superior efficacy compared with flecainide and dofetilide for the
was also found to prevent tachycardia-induced reductions in α1c-subunit expression. To the best of our knowledge, this is the first demonstration in the literature of the ability of a drug to prevent Ca2+-channel downregulation by atrial tachycardia.

Potential Significance
At present, AF is treated primarily with antiarrhythmic drugs that alter atrial electrical properties directly. Because the same agents affect ventricular electrophysiology, they risk promoting ventricular tachyarrhythmias and thereby directly increasing cardiac mortality. Pharmacological therapy to prevent remodeling is attractive because it would target the processes that promote the occurrence and maintenance of AF at a more fundamental level. In the present study, we found that amiodarone prevented tachycardia-induced atrial electrophysiological remodeling, in terms of both atrial electrical properties and ion-channel subunit expression. Amiodarone has Ca2+-, Na+-, and K+-channel blocking properties and is uniquely effective in clinical AF. We found amiodarone to be very effective in preventing experimental AF in the setting of atrial tachycardia remodeling, in contrast to the inefficacy of the Na+-channel blocker flecainide and the K+-channel blocker dofetilide. Highly selective L-type Ca2+-channel blockers have also been shown to be ineffective in the same model. Because atrial remodeling is the primary factor promoting AF in the dog model, the superior efficacy of amiodarone is very likely related to its antiremodeling actions. Since atrial remodeling is believed to contribute significantly to the occurrence and maintenance of AF in humans, the ability of amiodarone to prevent remodeling may play an important role in the superior clinical efficacy of the drug.

The precise mechanism of the antiremodeling action of amiodarone is unclear. Because both amiodarone and mibebradil are effective in remodeling and share T-type Ca2+-channel-inhibiting actions, it is tempting to speculate that T-type blockade plays a central role; however, confirmation with more selective agents is needed. In addition, although the other ion-channel blocking actions of amiodarone are insufficient to prevent remodeling, it is quite likely that they contribute to the antiarrhythmic effects of the drug in addition to antiremodeling actions.

Not only was amiodarone effective in preventing the development of remodeling, but it also reversed already established remodeling, as would occur in a patient given oral amiodarone after the onset of AF. The time-dependent reversal of remodeling may contribute to delayed pharmacological cardioversion in patients receiving oral amiodarone therapy for AF. The clinical value of amiodarone is limited by a wide range of side effects. The present demonstration of the potentially important role of remodeling prevention in the anti-AF properties of amiodarone may provide a useful paradigm for the development of novel compounds that combat AF by preventing atrial tachycardia–related remodeling.

Potential Limitations
The present study does not define the molecular mechanisms by which amiodarone affects remodeling. It is tempting to
speculate, given the T-type $I_C$-blocking actions of both amiodarone and mibefradil, that block of T-type Ca$^{2+}$ current plays a significant role. Nevertheless, both amiodarone and mibefradil have multiple additional actions. It therefore remains to be established whether their antiremodeling properties are a result of T-type $I_C$ blockade, alone or in combination with other channel-blocking actions, or whether other effects of the drugs are involved.

Relatively large doses of amiodarone, as applied clinically when rapid action is needed, were used to establish an effect at the onset of tachycycling. We believe that a similar result would occur with chronic oral dosing of the drug in man, but extrapolation must be cautious.

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