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 Ca$^{2+}$-current $\alpha_1$-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. AF treatment is suboptimal at present, and AF is the most common pathogenic factor for stroke in the elderly. Recent evidence indicates that disease- or arrhythmia-induced alterations in cardiac electrophysiology (electrical remodeling) are central in arrhythmia genesis, particularly for AF, which alters cardiac electrophysiology to promote its own maintenance. A key component of AF-related remodeling is downregulation of L-type Ca$^{2+}$-channel protein, which reduces atrial effective refractory period (ERP) and decreases physiological ERP rate adaptation.

Two recent controlled studies indicate that amiodarone is uniquely effective in treating AF; however, the underlying mechanistic basis is unknown. Mibebradil, a T-type Ca$^{2+}$-current blocker, prevents atrial electrical remodeling 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 Ca$^{2+}$-channel-blocking properties. Therefore we 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 Ca$^{2+}$-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.

Methods

Animal Model

Fifty-three mongrel dogs (18 to 39 kg) were used. Details of the model have been published previously. 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 (for studies of drug effects on atrial tachycardia associated AF) or 10 days (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 unpaced 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 α-chloralose (120 mg/kg IV, followed by 29.25 mg·kg⁻¹·h⁻¹) and ventilated. In atrial tachypacing dogs, the surface ECG was recorded to confirm maintained atrial and ventricular pacing and AV block. The atrial pacemaker was then deactivated. Body temperature was maintained at 37°C and the left femoral artery and both femoral veins were cannulated for pressure monitoring and drug administration. 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 ERP measurement and atrial vulnerability determination.

Final open-chest studies were performed during sinus rhythm; if AF occurred during surgery, we required normal rhythm to be restored before study. In all cases, this occurred spontaneously. To estimate the mean duration of AF, AF was induced (10-Hz burst pacing, 2 ms at 4× threshold stimuli) 10 times if AF duration was ≤20 minutes and 5 times if AF lasted between 20 and 30 minutes. AF lasting >30 minutes, which was considered persistent, was terminated by DC cardioversion, and 30 minutes was allowed before the experiment was continued. If persistent AF was induced on 2 occasions, no further AF inductions were performed, and mean AF duration was calculated on the basis of all AF episodes up to and including the second episode of sustained AF. Atrial vulnerability was defined as the percentage of sites in each dog at which AF (>1 second) could be induced by single extrastimuli. AF was defined as an atrial rhythm >400 bpm, with irregular atrial electrogram morphology and rate.

The ERP was measured at the LA appendage with 15 basic (S1) stimuli at basic cycle lengths (BCLs) of 150, 200, 250, 300, and 360 ms, followed by a premature (S2) stimulus, with ERP being the longest S1-S2 interval that failed to produce a response. The mean of 3 ERP values at each BCL was used for data analysis. In the case of a difference of ≥10 ms between measurements, 1 to 2 additional ERP measurements were obtained, and the mean of all determinations was used. Conduction velocity (CV) was measured in the LA and RA free walls as previously described. In addition to obtaining ERP measurements at 5 BCLs at the LA appendage, we measured atrial ERP at a BCL of 300 ms in 6 additional sites: the RA appendage, RA posterior wall, RA side of Bachmann’s bundle, LA posterior wall, LA inferior wall, and LA Bachmann’s bundle.

**L-Type Ca²⁺-Channel α₁C-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 (14 000g, 10 minutes, 4°C), and the soluble fraction 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 α₁C) 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/sufentanyl 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.
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 \((\text{Amio/A}^+)\). 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 \((A^+, \text{Figure 2, left})\). 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

Figure 2. Effects of study drugs on ERP changes caused by atrial remodeling. Left, Mean±SEM ERP as a function of BCL in each group. \( *P<0.05 \) vs control values. \( \dagger\dagger P<0.01 \) vs \( A^+ \). Right, ERP rate adaptation (ERP at BCL 360 ms minus value at 150 ms) for each group. \( ^\circ P<0.05 \), \( \circ P<0.01 \) vs CTL, \( \dagger P<0.01 \) vs \( A^+ \). Abbreviations as in Figure 1.

Figure 3. Effects on ERPs in different atrial regions. RAA, RAPW, and RABB indicate RA appendage, posterior wall, and Bachmann’s bundle, respectively; LAA, LAIW, LAPW, and LABB, LA appendage, inferior wall, posterior wall, and Bachmann’s bundle. Other abbreviations as in Figure 1.

** P<0.01 vs CTL \( \dagger P<0.01 \) drug vs \( A^+ \).

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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 decrease 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 α1c-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 Ca$^{2+}$-channel α1c-subunit (CaV1.2) 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 CaV1.2. 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 CaV1.2 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 CaV1.2 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
prevention of AF associated with atrial tachycardia remodeling. Amiodarone prevented tachycardia-induced changes in atrial ERP and L-type Ca\(^{2+}\)-channel \(\alpha_{1c}\)-subunit protein expression, suggesting that the prevention of atrial remodeling contributed significantly to the efficacy of the drug.

Comparison With Previous Studies of Drug Effects on Atrial Tachycardia Remodeling

To date, efforts to develop drugs that affect the atrial remodeling process have been largely fruitless. The physiological consequences of relatively brief periods (up to several hours) of atrial tachycardia, believed to be functional, can be prevented by several drugs, including L-type Ca\(^{2+}\)-channel blockers, renin-angiotensin system antagonists, and Na\(^+-\),H\(^+-\) exchange inhibitors.\(^{13-20}\) Longer-term consequences, caused by changed gene expression, are not suppressed by any of these compounds.\(^{12,14,21}\) The only agent that has been found to prevent ERP changes and AF promotion by longer-term atrial tachycardia is mibefradil,\(^{13,14}\) which is no longer available.

Furthermore, although mibefradil appears to attenuate strongly atrial tachycardia–induced in vivo electrophysiological changes, a direct interaction has not been confirmed by analysis of effects on ion-channel expression. In the present study, amiodarone was found to strongly attenuate the electrophysiological changes caused by atrial tachycardia and was also found to prevent tachycardia-induced reductions in \(\alpha_{1c}\)-subunit expression. To the best of our knowledge, this is the first demonstration in the literature of the ability of a drug to prevent Ca\(^{2+}\)-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.\(^{22}\) 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.\(^{23}\) 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 Ca\(^{2+}\)-, Na\(^+\)-, and K\(^+\)-channel blocking properties\(^{24}\) 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 ineffectivity of the Na\(^+\)-channel blocker flecainide and the K\(^+\)-channel blocker dofetilide. Highly selective L-type Ca\(^{2+}\)-channel blockers have also been shown to be ineffective in the same model.\(^{12,21}\) 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,\(^{5,6,25}\) 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 Ca\(^{2+}\)-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 $I_{Ca}$-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 $I_{Ca}$ 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 tachypacing. 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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