Amiodarone-Induced Postrepolarization Refractoriness Suppresses Induction of Ventricular Fibrillation

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ABSTRACT
It is still incompletely understood why amiodarone is such a potent antiarrhythmic drug. We hypothesized that chronic amiodarone treatment produces postrepolarization refractoriness (PRR) without conduction slowing and that PRR modifies the induction of ventricular arrhythmias. In this study, the hearts of 15 amiodarone-pretreated (50 mg/kg p.o. for 6 weeks) rabbits and 13 controls were isolated and eight monophasic action potentials were simultaneously recorded from the epicardium and endocardium of both ventricles. Steady-state action potential duration (APD), conduction times, refractory periods, and dispersion of action potential durations were determined during programmed stimulation and during 50-Hz burst stimuli, and related to arrhythmia inducibility. Amiodarone prolonged APD by 12 to 15 ms at pacing cycle lengths of 300 to 600 ms (p < 0.05) but did not significantly increase conduction times or dispersion of APD. Amiodarone prolonged refractoriness more than action potential duration, resulting in PRR (refractory period – APD at 90% repolarization, 14 ± 10 ms, p < 0.05 versus controls), PRR curtailed the initial sloped part of the APD restitution curve by 20%. During burst stimulation, pronounced amiodarone-induced PRR (40 ± 15 ms, p < 0.05 versus controls) reduced the inducibility of ventricular arrhythmias (p < 0.05 versus controls). Furthermore, in 35% of bursts only monomorphic ventricular tachycardias and no longer ventricular fibrillation were inducible in amiodarone-treated hearts (p < 0.05 versus controls). Chronic amiodarone treatment prevents ventricular tachycardias by inducing PRR without much conduction slowing, thereby curtailing the initial part of APD restitution. PRR without conduction slowing is a desirable feature of drugs designed to prevent ventricular arrhythmias.

The need to prevent frequent appropriate discharges of implantable defibrillators, the side effects of long-term defibrillator therapy, and the increasing economic constraints in several health care systems have reemphasized the need for antiarrhythmic agents that prevent ventricular arrhythmias. Chronic amiodarone treatment reduces recurrent ventricular tachycardias (Connolly, 1999) and, in contrast to other antiarrhythmic agents, including potassium channel blockers, does not increase mortality or sudden death rates (Echt et al., 1991; Singh et al., 1995; Waldo et al., 1996; Wyse et al., 2001). Therefore, amiodarone is one of the few remaining treatment options to prevent recurrent ventricular arrhythmias (Connolly, 1999). Although amiodarone blocks multiple ion currents in the heart (Kamiya et al., 2001; Maltsev et al., 2001), the electrophysiological effects by which amiodarone exerts this unique antiarrhythmic action are not well understood.

A series of premature stimuli applied at the shortest possible coupling interval allow earlier capture of each consecutive stimulus. This shortening of the effective refractory period (ERP) is not only caused by rate-dependent decrease in action potential duration (APD) (Franz et al., 1988) but also because each additional premature stimulus captures the myocardium at an earlier repolarization level than the previous one (Koller et al., 1995). This phenomenon called “progressive encroachment” or “facilitated excitability during repetitive extrastimulation” (Koller et al., 1995) is accompanied by a progressive slowing of impulse conduction velocity, a predictor of ventricular inducibility (Koller et al., 1995; Kirchhof et al., 1998). Sodium channel

ABBREVIATIONS: ERP, effective refractory period; APD, action potential duration; PRR, postrepolarization refractoriness; MAP, monophasic action potential, BCL, basic cycle length.
blocks can prevent progressive encroachment by prolonging refractoriness beyond repolarization, an effect that has been called postrepolarization refractoriness (PRR; Kirchhof et al., 1998). We have previously shown that PRR induced by sodium channel blockers inhibits the induction of ventricular fibrillation, but in the case of sodium channel blockers, this effect is offset by conduction slowing, a known proarrhythmic factor that facilitates induction of monomorphic ventricular tachycardias (Kirchhof et al., 1998).

Acute administration of amiodarone can induce PRR in isolated cells (Mason et al., 1983; Varro et al., 1985; Yabek et al., 1986; Nanas and Mason, 1995). Based on these findings and on our previous studies (Kirchhof et al., 1998), we hypothesized that amiodarone may induce PRR without much conduction slowing and that this electrophysiological effect prevents the induction of ventricular arrhythmias. Because the electrophysiological effects of chronic amiodarone treatment differ from its acute effects (Mason et al., 1983; Varro et al., 1985; Yabek et al., 1986; Nanas and Mason, 1995), we used a model of chronic amiodarone treatment to measure action potential durations, effective refractory periods, PRR, and conduction times in the intact heart.

Materials and Methods

Experimental Preparation and Data Acquisition. The study conformed with the Guide for the Care and use of Laboratory Animals published by the National Institutes of Health (NIH publication 85-23, revised 1986). Fifteen male New Zealand White rabbits (mean body weight 3.9 ± 0.5 kg) received oral amiodarone treatment (50 mg/kg b.wt./day) for 6 weeks. The drug was mixed into the normal food. Thirteen rabbits of comparable weight and equal sex served as controls. After the end of the treatment period, the hearts were isolated and retrogradely perfused via the aorta on a modified vertical Langendorff apparatus using a 37°C warm, oxygenated modified Krebs-Henseleit solution. Details of the isolated heart setup have been described previously (Kirchhof et al., 1998, 2003). In brief, eight monophasic action potential (MAP)-pacing combination catheters were simultaneously placed onto the epicardium of both ventricles to monitor left ventricular pressure. A volume-conducted six-lead ECG was recorded from a solution-filled tissue bath (Kirchhof et al., 1998). All data were acquired using a 24-channel EP lab system (EP system version 2.51; Bard Electrophysiology, Unterschleißheim, Germany). The atrioventricular node was crushed to avoid pacing at slow ventricular rates.

Electrophysiological Protocol. One of the left ventricular epicardial MAP catheters was used for pacing and burst stimulation. The pacing threshold was checked repetitively during the stimulation protocol. All pacing stimuli were of 2-ms duration. First, the ventricle was paced at twice diastolic threshold for >1 min at 200-, 300-, 400-, and 600-ms pacing cycle length, respectively, to determine steady-state action potential durations and conduction times. Programmed stimulation was performed using up to three extra stimuli at 400- and 600-ms basic drive cycle length. The coupling interval of the extra stimulus was decreased in steps of 5 ms. The effective refractory period was defined as the longest coupling interval not eliciting a premature response and was determined twice for each extra stimulus. For determination of the ERP of S3, the coupling interval of the previous extra stimulus was set at ERP (S2) plus 5 ms.

Burst Stimulation. To determine the vulnerability of the ventricles to extremely premature stimulation, the heart was stimulated for 5 s using 50-Hz burst stimuli at 2, 3, and 5 times diastolic threshold, and at maximal output strength (corresponding to 8–20 times diastolic threshold). This stimulation frequency is the most effective to induce ventricular arrhythmias in this model (Kirchhof et al., 1998). Each burst stimulus was repeated three times to assess the probability of arrhythmia induction. Burst stimulation allows for multiple consecutive premature stimuli as close to refractoriness as possible. For assessment of arrhythmia inducibility, we chose burst stimulation and not conventional programmed stimulation because this technique can be repeated multiple times within a short time period (Kirchhof et al., 1998). The entire stimulation protocol was performed via a single MAP catheter to be able to compare the measurements during programmed stimulation and during burst stimulation.

Data Analysis. All data were exported on a personal computer and analyzed using a semiautomatic computer program for analysis of action potential duration (Franz et al., 1995). The program was used to determine action potential durations in each MAP recording at 50, 70, and 90% repolarization (APD50, APD70, and APD90), and conduction times during steady-state pacing and during programmed stimulation for the construction of APD restitution curves. Conduction times were measured as the interval from the pacing stimulus to the fastest part of the upstroke in each of the eight MAP recordings. The timing of the MAP upstroke was determined digitally under visual control of an experienced observer. The mean and maximal conduction times were calculated over all MAP recordings in every beat analyzed (Kirchhof et al., 1998). Dispersion

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<td>Mean and maximal conduction times (CT) and PRR during pacing at difference pacing cycle lengths (CL) in control hearts (Base) and in amiodarone-treated hearts (Amio)</td>
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**Fig. 1.** Amiodarone prolongs steady-state action potential durations. Mean steady-state APD, calculated as mean of eight MAP measurements for each heart, are shown at 50% (APD50), 70% (APD70), and 90% repolarization (APD90) for untreated hearts (open dots) and amiodarone-pretreated hearts (filled dots) at pacing cycle lengths from 200 to 600 ms (abscissa). Amiodarone prolonged APD at all repolarization levels at cycle lengths from 300 to 600 ms (all p < 0.01).
of APD was calculated as the difference between minimal and maximal APD in the eight MAP recordings. PRR was calculated as ERP minus APD90. During burst stimuli and programmed stimulation, PRR was manually measured in the MAP recording that was used for pacing at a paper speed of 200 mm/s as the interval from repolarization of the previous action potential to below 90% to the stimulus eliciting the following action potential (Kirchhof et al., 1998). Induced arrhythmias were classified as monomorphic ventricular tachycardia or ventricular fibrillation based on ECG and MAP characteristics. Arrhythmias were defined as sustained if they lasted longer than 15 s and were terminated by a defibrillator (CPI Ventak 2815; Guidant Corp., Giessen, Germany) that delivered monophasic shocks through two defibrillation electrodes by a defibrillator (CPI Ventak 2815; Guidant Corp., Giessen, Germany).

After the end of the experiment, the myocardial tissue below each MAP catheter was excised to assess amiodarone tissue levels using a high-performance liquid chromatography amiodarone assay (n = 7 amiodarone-treated hearts, n = 2 untreated hearts, 6–8 specimens/heart; Latini et al., 1983; Laer et al., 1997). In brief, about 200 mg of frozen myocardium and internal standard (trifluoperazine) were homogenized using potassium acetate buffer (2 M, pH 4.1) and extracted using diethyl ether. After centrifugation, the supernatant was evaporated to dryness, and the residues were reconstituted in a mixture of 80% acetonitrile and 20% 0.1 M potassium phosphate buffer (pH 5). The linearity range of amiodarone is between 1.3 and 101 μg/g using this method.

**Statistics.** Continuous values were compared between groups using univariate tests. Absence of arrhythmia inducibility was compared between the two experimental groups using a modified Kaplan Meier analysis with burst stimulus strength (2, 3, and 5 times, or maximal diastolic threshold) used as the continuous parameter. All tests were performed using an SPSS software package (SPSS Science, Chicago, IL). Two-sided p values < 0.05 were considered significant. All values are given in the text as mean ± standard deviation unless indicated otherwise.

**Results**

**Steady-State Action Potential Durations and Tissue Concentrations.** Amiodarone prolonged APD at 300- to 600-ms basic cycle lengths (BCLs) and at all repolarization levels analyzed (Fig. 1). Dispersion of APD was not changed by amiodarone (maximal difference 6 ms, all p > 0.2). Alternans of APD did not occur at pacing cycle lengths from 200 to 600 ms. Conduction times were not significantly different in amiodarone-treated hearts compared with baseline hearts, although there was a trend toward a slight prolongation of conduction times in amiodarone-treated hearts (p = 0.07–0.14, Table 1). Conduction times during programmed stimulation did not increase in amiodarone-treated hearts (see below). Myocardial amiodarone tissue levels ranged from 5.1 ± 0.9 to 13.1 ± 2.3 μg of amiodarone per gram of myocardium. The mean amiodarone tissue concentration was 7.9 ± 0.6 μg of amiodarone per gram of myocardium, comparable with myocardial tissue concentrations in human hearts during chronic amiodarone treatment (Candinas et al., 1998; Anastasiou-Nana et al., 1999). Amiodarone tissue concentrations were not different between right and left ventricular specimens.

**APD Restitution Curve, Refractoriness, and Conduction Times.** During programmed stimulation, the initial portion of the APD restitution curve was significantly curtailed by ~20% of its total duration in amiodarone-treated hearts (Fig. 2, p < 0.05). This curtailing of the APD restitution curve was due to postrepolarization refractoriness (see below) and resulted in a lesser degree of APD shortening during programmed stimulation [600-ms BCL: shortest APD after S2 amiodarone 150 ± 9 ms; baseline 135 ± 15 ms, p < 0.05; 400-ms BCL: amiodarone 121 ± 16 ms; baseline 110 ± 19 ms, p = 0.07]. The remaining portion of the restitution curve was not significantly changed by amiodarone treatment (Fig. 2, A and B). Upon inspection of the restitution curves, a small upward deviation was noted in amiodarone-treated hearts for long S2 coupling intervals at a pacing cycle length of 600 ms (Fig. 2B), probably caused by the potassium
were induced in 35% of bursts in amiodarone-treated hearts (white). Instead, slower monomorphic ventricular tachycardias (black) stimulation. Amiodarone reduced the likelihood of ventricular fibrillation and monomorphic ventricular tachycardias induced by burst stimulation protocol (mean PRR during bursts irrespective of stimulus strength: amiodarone 40 ± 15 ms versus controls 22 ± 13 ms, p < 0.05). Presence of PRR during burst stimuli prevented induction of ventricular fibrillation (Fig. 5C). Higher burst stimulus strengths reduced PRR and reverted PRR to progressive encroachment (Fig. 5, B and C), concurrent with increased arrhythmia inducibility (Fig. 3A).

Burst stimulation episodes that did not induce arrhythmias showed marked PRR (Fig. 5D). Induction of ventricular fibrillation, in contrast, was associated with progressive encroachment, or lack of PRR. Of note, the bursts that induced monomorphic ventricular tachycardias showed PRR that was not significantly different from bursts not inducing arrhythmias. Thus, amiodarone-induced PRR prevented induction of ventricular fibrillation in favor of no arrhythmias at all or in favor of hemodynamically better tolerated monomorphic ventricular tachycardias.

**Discussion**

**Main Findings.** Chronic amiodarone treatment induced marked PRR (Figs. 3 and 5). Amiodarone-induced PRR prevented the induction of ventricular arrhythmias and furthermore reduced the incidence of ventricular fibrillation in favor of monomorphic ventricular tachycardias (Figs. 3–5). PRR prevented excitation during the vulnerable period and curtailed the initial, steep portion of the APD restitution curve (Figs. 2 and 5). PRR without conduction slowing is a desirable effect of drugs designed to prevent ventricular arrhythmias.

**Relation of Refractoriness and Repolarization.** The effective refractory period is known to relate to repolarization levels between 75 and 85% in different animal models and in human (Franz et al., 1988, 1990; Lee et al., 1992). Premature stimulation alters this fixed relationship between APD and refractory period (Koller et al., 1995; Kirchhof et al., 1998): closely coupled extra stimuli shorten the refractory period of the premature responses due not only to a parallel decrease in the concomitant APD but also because premature excitation is possible at increasingly less complete repolarization levels (progressive encroachment; Davidenko and Antzele-
attributable to the development of PRR, which prevents stimulation times during programmed stimulation in the same experimental model (Kirchhof et al., 1998) and may be prevented by the sodium channel blocker propafenone (Kirchhof et al., 1998). In our previous study, propafenone-induced PRR prevented ventricular fibrillation, but this apparently antiarrhythmic effect was offset by marked conduction slowing that promoted monomorphic ventricular tachycardias (Kirchhof et al., 1998). Chronic amiodarone treatment, in contrast, induced PRR without a high degree of stimulation-induced conduction slowing in this study, and reduced the inducibility of ventricular arrhythmias (Figs. 3 and 4). PRR was present both during programmed stimulation and during burst stimulation in amiodarone-treated hearts. These findings provide direct evidence that PRR has antiarrhythmic effects in the intact heart in the absence of conduction slowing.

**Conduction Times.** In this model, we assessed conduction times during programmed stimulation in eight simultaneous MAP recordings that were equally spread throughout the right and left ventricular epicardium, a surrogate parameter for conduction velocity in this model (Kirchhof et al., 1998). We found a trend toward longer conduction times in amiodarone-treated hearts during steady-state pacing, compatible with the sodium channel-blocking effect of amiodarone (Mason et al., 1983; Maruyama et al., 1995). Conduction times were not significantly prolonged in amiodarone-treated hearts, probably due to the intrinsic variability of conduction times measured between different hearts by equally spread MAP recordings. Noteworthy is the fact that amiodarone did not enhance the increase in conduction times associated with programmed stimulation. This is in contrast to slowly dissociating sodium channel blockers that markedly slow conduction times during programmed stimulation in the same experimental model (Kirchhof et al., 1998) and may be attributable to the development of PRR, which prevents stimulation during relative refractoriness. Lack of conduction slowing during premature stimulation could prevent wave-length shortening and induction of monomorphic ventricular tachycardias (Kirchhof et al., 1998).

**How Could PRR Prevent Arrhythmia Induction?** PRR allows for full recovery of voltage-dependent sodium channels during the refractory period (Maruyama et al., 1995), as reflected by relatively rapid upstroke velocities of action potentials after an extra stimulus in isolated tissue preparations that are not different from upstroke velocities during fixed frequent pacing (Pallandi and Campbell, 1987). Previously, we hypothesized that this effect may reduce stimulation-induced conduction slowing and thereby prevent induction of ventricular tachyarrhythmias (El-Sherif, 1991; Koller et al., 1995; Kirchhof et al., 1998). In this study, however, the degree of stimulation-induced conduction slowing was similar at baseline and in amiodarone-treated hearts, potentially due to the inactivated sodium channel-blocking effect of amiodarone (Pallandi and Campbell, 1987; Maruyama et al., 1995). Preventing stimulation-induced conduction slowing can therefore not fully explain the antiarrhythmic effect of PRR. Amiodarone-induced PRR must exert other antiarrhythmic effects. These may include curtailing of APD restitution and prevention of excitation during the vulnerable period.

Due to loss of excitability during the late repolarization phase, the initial portion of the APD restitution curve was eliminated in amiodarone-treated hearts, resulting in a shorter steep portion of the APD restitution curve (Fig. 2, C and D). This effect may prevent or reduce alternans of action potential duration after premature stimuli or during high heart rates (Fox et al., 2002), a known proarrhythmic factor that precedes wave front breakup and induction of ventricular fibrillation (Weiss et al., 2000; Ohara et al., 2001). Due to the lengthy stimulation protocol for induction of arrhythmias, we could not directly quantify APD alternans in the present study. APD alternans occur, however, when a steep slope of APD restitution is present over a long range of coupling intervals (Weiss et al., 2000; Fox et al., 2002). Amiodarone-
Amiodarone curtailed this steep portion of APD restitution in our study. Of note, the remainder of the restitution curve was not altered by amiodarone, suggesting that the curtailed portion of the restitution curve is a direct consequence of PRR. Progressive encroachment of excitation will allow premature activations to occur during the vulnerable period. Activation wave fronts induced during the vulnerable period will then encounter conduction slowing due to the relative refractoriness of adjacent tissue, and functional block due to dispersion of refractoriness. These are potential contributors to functional conduction block (Frazier et al., 1989), wave front breakup, and initiation of ventricular fibrillation (Weiss et al., 2000; Ohara et al., 2001). Amiodarone-induced PRR prevents excitation during the vulnerable period and may thereby avoid induction of reentry by premature excitations. Amiodarone-induced PRR may also contribute to longer cycle lengths and an enlarged core of spiral waves during ventricular fibrillation in amiodarone-treated swine (Omichi et al., 2002).

Comparison to Other Antiarrhythmic Agents. Potassium channel blockers prolong both repolarization and refractoriness to a similar extent, and therefore do not induce PRR (Kirchhof et al., 1996; Zabel et al., 1997). Sodium channel blockers induce PRR but markedly slow conduction velocity (Franz and Costard, 1988; Kirchhof et al., 1998). These effects may explain why both sodium and “pure” potassium channel blockers have more pro- than antiarrhythmic effects in clinical trials (Echt et al., 1991; Singh et al., 1995; Waldo et al., 1996; Wyse et al., 2001). PRR will be present when an action potential-prolonging drug, e.g., sotalol, is combined with a sodium channel blocker, e.g., mexiletine. PRR could therefore also contribute to the antiarrhythmic effects of such combinations of antiarrhythmic drugs (Breithardt et al., 1981; Chezalviel-Guilbert et al., 1995; Lee et al., 1997).

Methodological Considerations. Our data pertain to ventricular arrhythmias induced by multiple electrical stimuli and their prevention by antiarrhythmic agents in the intact rabbit heart. Although this experimental setup quantifies arrhythmia inducibility, combined with multisite assessment of conduction times, action potential durations, and refractoriness, the results cannot be directly transferred to the clinical setting in which a variety of underlying cardiac diseases and autonomic influences form additional anti- and proarrhythmic factors. Some data suggest that amiodarone may have antiarrhythmic effects in ischemic tissue or in hearts that survived a myocardial infarction (Manning et al., 1995; Aimond et al., 2000). Further studies may determine the effect of amiodarone on PRR in hearts with acute ischemia or myocardial infarction, and whether these results also pertain to arrhythmias provoked by other techniques.

Proarrhythmic effects associated with other mechanisms of arrhythmia induction than rapid ventricular stimulation and functional reentry, e.g., after depolarizations and repolarization-related arrhythmias, were not assessed in this study. Amiodarone did not increase dispersion of repolarization in this and other studies (Sicouri et al., 1997; Zabel et al., 1997), in keeping with its low proarrhythmic potential (Haverkamp et al., 2000).

Others have previously demonstrated that the acute effects of amiodarone include a prolongation of refractoriness beyond repolarization in isolated rabbit papillary muscle (Pallandi and Campbell, 1987; Maruyama et al., 1995). Maruyama et al. (1995) already suggested that the sodium channel-blocking effect of amiodarone might be one of the reasons why this drug is an effective antiarrhythmic agent. Our
study demonstrates that PRR mediates the antiarrhythmic efficacy of chronic amiodarone treatment in the intact heart.

**Implications.** In contrast to slowly dissociating sodium channel blockers and the pure potassium channel ($I_{Kp}$) blocker sotalol (Echt et al., 1991; Singh et al., 1995; Waldo et al., 1996; Wyse et al., 2001), amiodarone is the only antiarrhythmic drug whose antiarrhythmic potential is not offset by proarrhythmic effects in patients (Singh et al., 1995; Wyse et al., 2001). PRR can explain this unique antiarrhythmic efficacy of amiodarone, whereas lack of conduction slowing (this study) and uniform action potential prolongation (Siouri et al., 1997) may explain the low proarrhythmic potential of the drug (Singh et al., 1995; Wyse et al., 2001). The ideal antiarrhythmic agent has yet to be designed. Such a compound should eliminate premature responses by producing PRR, without interfering with normal excitation. So far, only amiodarone approximates these criteria.

**Acknowledgments**

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**References**


