Amiodarone-Induced Post-Repolarization Refractoriness Suppresses Induction of Ventricular Fibrillation

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Abbreviations:

APD  action potential duration
ECG  electrocardiogram
ERP  effective refractory period
MAP  monophasic action potential
mVT  monomorphic ventricular tachycardia
PRR  post-repolarization refractoriness
VF   ventricular fibrillation

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ABSTRACT

Objective. It is still incompletely understood why amiodarone is such a potent antiarrhythmic drug. We hypothesized that chronic amiodarone treatment produces post-repolarization refractoriness (PRR) without conduction slowing and that PRR modifies the induction of ventricular arrhythmias.

Methods. The hearts of 15 amiodarone-pretreated (50mg/kg p. o. for 6 weeks) rabbits and 13 controls were isolated and 8 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.

Results. Amiodarone prolonged APD by 12-15ms at pacing cycle lengths of 300-600ms (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±10ms, p<0.05 vs. controls). PRR curtailed the initial sloped part of the APD restitution curve by 20%. During burst stimulation, pronounced amiodarone-induced PRR (40±15ms, p<0.05 vs. controls) reduced the inducibility of ventricular arrhythmias (p<0.05 vs. controls). Furthermore, in 35% of bursts only monomorphic ventricular tachycardias and no longer ventricular fibrillation were inducible in amiodarone-treated hearts (p<0.05 vs. controls).

Conclusions. Chronic amiodarone treatment prevents ventricular tachycardias by inducing PRR without much conduction slowing, thereby curtailing of 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 re-emphasized the need for antiarrhythmic agents that prevent ventricular arrhythmias. Chronic amiodarone treatment reduces recurrent ventricular tachyarrhythmias (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 VT inducibility (Koller et al., 1995; Kirchhof et al., 1998). Sodium channel blockers can prevent progressive encroachment by prolonging refractoriness beyond repolarization, an effect that has been called post-repolarization 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 electrophysiologic effect prevents the induction of ventricular arrhythmias. As 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.
METHODS

Experimental preparation and data acquisition. The study conformed with the *Guide for the Care and use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Fifteen white male New Zealand rabbits (mean body weight 3.9 ± 0.5 kg) received oral amiodarone treatment (50 mg / kg body weight per 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; Kirchhof et al., 2002). In brief, a total of 8 monophasic action potential-pacing combination catheters (MAP) were simultaneously placed onto the epicardium of both ventricles and into the right ventricular cavity. We used MAP combination catheters as they allow for stimulation and recording of an action potential at the same site. A custom-designed latex balloon was connected to a pressure transducer and placed into the left ventricle to monitor left ventricular pressure. A volume-conducted 6-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 (Bard Electrophysiology, EP System version 2.51). The AV node was crushed to allow 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 minute 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 3 extra stimuli at 400 ms 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 five seconds using 50Hz burst stimuli at twice, 3 times, 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 as 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 in order to be able to compare the measurements during programmed stimulation and during burst stimulation.

**Data analysis.** All data were exported on a personal computer system and analyzed using a semi-automatic 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 8 MAP recordings. The timing of the MAP upstroke was determined digitally under visual control of an experienced observer. The mean and maximal conduction time was calculated over all MAP recordings in every beat analyzed (Kirchhof et al., 1998). Dispersion of APD was calculated as the difference between minimal and maximal APD in the 8 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 seconds and were terminated by a defibrillator (CPI Ventak 2815) which delivered monophasic shocks through two defibrillation electrodes placed in the tissue bath.

After the end of the experiment, the myocardial tissue below each MAP catheter was excised to assess amiodarone tissue levels using an HPLC amiodarone assay (n=7 amiodarone-treated hearts, n=2 untreated hearts, 6-8 specimen per heart, (Latini et al., 1983; Laer et al., 1997)). In brief, about 200mg of frozen myocardium and internal standard (trifluoperazine) were homogenized using potassium acetate buffer (2M, 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% 0.1M potassium phosphate buffer (pH 5). The linearity range of amiodarone is between 1.3 and 101µg/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 (twice, three times, 5 times, or maximal diastolic threshold) used as the continuous parameter. All tests were performed using an SPSS software package. 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 – 600 ms basic cycle lengths and at all repolarization levels analyzed (Figure 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 – 600 ms. Conduction times were not significantly different in amiodarone-treated hearts compared to baseline hearts, although there was a trend towards a slight prolongation of conduction times in amiodarone-treated hearts (p = 0.07 - 0.14, Table). 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 amiodarone/g myocardium. The mean amiodarone tissue concentration was 7.9 ± 0.6 µg amiodarone/g myocardium, comparable to 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 (Figure 2, p < 0.05). This curtailing of the APD restitution curve was due to post-repolarization 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 (Figure 2A-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 600ms (Figure 2B), probably caused by the potassium channel-blocking properties of amiodarone. In contrast to the curtailing of the initial part of the restitution curve, these subtle changes did not reach statistical significance, thereby
demonstrating the relevance of the initial portion of the restitution curve for the antiarrhythmic action of amiodarone.

Conduction times increased during premature stimulation both in controls and in amiodarone-treated hearts. Neither mean conduction times nor the increase in conduction time during programmed stimulation was different between amiodarone-treated hearts and controls (conduction times at BCL = 600 ms: amiodarone increase from 38 ± 1 ms (at diastolic intervals > 150 ms) to 54 ± 4 ms (refractory period + 5 ms); baseline increase from 36 ± 1 ms to 54 ± 4 ms; BCL = 400 ms: amiodarone increase from 41 ± 2 ms to 56 ± 3 ms, baseline increase from 32 ± 3 ms to 50 ± 3 ms). Amiodarone induced PRR during programmed stimulation (p < 0.05 versus base for S1 = 600 ms, Table).

**Ventricular arrhythmias during burst stimulation.** A total of 1,189 burst stimulation episodes were analyzed. In 282 episodes (24%), sustained ventricular arrhythmias were induced. Arrhythmia induction was more likely at higher stimulus strengths (Figure 3A). Amiodarone reduced arrhythmia inducibility at all stimulus strengths (Figure 3A, p < 0.05). The number of arrhythmia-free hearts was higher in the amiodarone-treated group (Figure 3B). Furthermore, 35% of burst stimuli induced monomorphic ventricular tachycardias instead of ventricular fibrillation in amiodarone-treated hearts (Figure 3C, Figure 4, p < 0.05). During monomorphic tachycardias, the left ventricle still generated pressure, suggesting a residual systolic left ventricular function (Figure 4, “Amio”).

**Amiodarone-induced PRR prevents arrhythmia induction.** Amiodarone induced PRR during programmed stimulation (Table, Figure 5A). During burst stimulation, PRR was more pronounced than during programmed stimulation (Mean PRR during bursts irrespective of stimulus strength: Amiodarone 40 ± 15 ms vs. controls 22 ± 13 ms, p < 0.05). Presence of PRR during burst stimuli prevented induction of ventricular fibrillation (Figure 5C). Higher burst stimulus strengths reduced PRR and reverted PRR to progressive encroachment (Figure 5B & 5C), concurrent with increased arrhythmia inducibility (Figure 3A).
Burst stimulation episodes that did not induce arrhythmias showed marked PRR (Figure 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 (Figures 3&5). Amiodarone-induced PRR prevented the induction of ventricular arrhythmias and furthermore reduced the incidence of ventricular fibrillation in favor of monomorphic ventricular tachycardias (Figures 3-5). PRR prevented excitation during the vulnerable period and curtailed the initial, steep portion of the APD restitution curve (Figures 2&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 man (Franz et al., 1988; Franz et al., 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 Antzelevitch, 1986; Koller et al., 1995; Kirchhof et al., 1998)). Progressive encroachment of excitation was related to the induction of ventricular tachyarrhythmias in our study (Figure 5C) and in previous studies (Davidenko and Antzelevitch, 1986; Koller et al., 1995; Kirchhof et al., 1998).

Post-repolarization refractoriness (PRR). Progressive encroachment of excitation (Koller et al., 1995) can 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 which promoted monomorphic ventricular tachycardias (Kirchhof et al., 1998). Chronic amiodarone treatment, in contrast, induced PRR without a high degree of conduction slowing in this study, and reduced the inducibility of ventricular arrhythmias (Figures 3&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 8 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 towards 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 monomorph 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 following an extra stimulus in isolated tissue preparations that are not different from upstroke velocities during fix 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 (Figure 2C-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 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 initial portion of the restitution curve is a direct consequence of PRR.

PRR prevents electrical stimulation during the so-called vulnerable period of the heart which is delineated by the dispersion of action potential durations from 70% to 90% repolarization (Kirchhof et al., 1996). A single strong electrical field stimulus almost inevitably induces ventricular fibrillation when it is applied during this vulnerable period at a certain range of shock strengths. In this setting, ventricular fibrillation is induced by causing micro-reentry in regions of slow conduction and functional block (Frazier et al., 1989). 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 break up 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. While this experimental setup quantifies arrhythmia inducibility, combined with multi-site 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; Aimon 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. afterdepolarizations 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. (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_{Kr}) 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, while lack of conduction slowing (this study) and uniform action potential prolongation (Sicouri 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.

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FOOTNOTES

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LEGENDS FOR FIGURES

Figure 1. Amiodarone prolongs steady-state action potential durations. Mean steady-state APD, calculated as mean of 8 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 200-600ms (abscissa). Amiodarone prolonged APD at all repolarization levels at cycle lengths from 300-600ms (all p<0.01).

Figure 2: Amiodarone curtails APD restitution curves at 600 ms basic cycle length (BCL) and 400 ms BCL. Action potential duration of the premature response (APD) is given on the ordinate, and the coupling interval of the extra stimulus on the abscissa.
A: APD restitution curve at 400 ms basic cycle length (BCL).
B: APD restitution curve at 600 ms basic cycle length (BCL). Amiodarone curtailed the initial, steep portion of the APD restitution curve.
C&D: Initial, sloped portion of the APD restitution curve at 400 ms BCL (C) and at 600 ms BCL (D). Horizontal black bars highlight the curtailed initial portion of the APD restitution curve in amiodarone-treated hearts.

Figure 3: Amiodarone prevents arrhythmia induction by burst stimulation.
A: Probability of arrhythmia induction by 50Hz burst stimuli at different stimulus strengths (2x-maximal diastolic threshold) in amiodarone-treated hearts and in controls. Amiodarone reduced the incidence of arrhythmia induction at all stimulus strengths.
B: Kaplan-Meier curve of arrhythmia-free burst stimulation for amiodarone-treated hearts and controls. Amiodarone increased number of arrhythmia-free hearts during the burst stimulation protocol (p < 0.05).
C: Proportion of ventricular fibrillation and monomorphic ventricular tachycardias induced by burst stimulation. Amiodarone reduced the likelihood of ventricular fibrillation (white). Instead,
slower monomorphic ventricular tachycardias (black) were induced in 35% of bursts in amiodarone-treated hearts (p <0.05).

**Figure 4:** Ventricular tachyarrhythmias induced by burst stimulation (stimuli not shown) as recorded by the volume-conducted ECG (I-aVF), eight simultaneous MAP recordings (MAP 1-8), and left ventricular pressure (LVP). Calibration bars indicate 100ms (horizontal) and 5 mV or 50mmHg (vertical). *Left panel:* Induction of ventricular fibrillation at baseline (Base). The left ventricle stops pumping. *Right panel:* Induction of a monomorphic ventricular tachycardia in an amiodarone-treated heart (Amio). The left ventricle generates systolic pressure during the tachycardia (LVP recording). *Bottom panel:* Schematic drawing of the experimental setup with the 8 MAP catheters in their recording positions.

**Figure 5:** Amiodarone-induced post-repolarization refractoriness (PRR) relates to prevention of arrhythmia induction.

*A:* Example of Amiodarone-induced PRR recorded in a monophasic action potential during programmed stimulation with 2 premature stimuli stimulated at the shortest possible coupling interval (ERP + 5 ms). Horizontal black bars indicate intervals of PRR in the amiodarone-treated heart.

*B, upper panel:* Typical example of encroachment of excitation during burst stimulation at maximal stimulus strength without amiodarone treatment. Upward arrows indicate take-off potentials of the next action potential. *Lower panel:* Typical example of PRR during burst stimulation at maximal stimulus strength in an amiodarone-treated heart. Horizontal bars indicate intervals of PRR. Calibration bars indicate 5 mV and 100 ms (A and B).

*C:* Mean PRR during burst stimulation in amiodarone-treated and control hearts split by stimulus strength (abscissa), and for all bursts (rightmost column). PRR decreased with increasing stimulus strength, related to a higher probability of arrhythmia induction (compare Figure 3A).
$D$: Mean PRR (ms) during bursts inducing ventricular fibrillation (VF), monomorphic ventricular tachycardias (mVT), or no arrhythmia (No Arrh). PRR was present when no arrhythmia was induced, less PRR when mVT was induced. Progressive encroachment was associated with induction of VF. Asterisks (*) indicate significant differences between groups.
TABLE

Mean and maximal conduction times (CT) and post-repolarization refractoriness (PRR) during pacing at difference pacing cycle lengths (CL) in control hearts (Base) and in amiodarone-treated hearts (Amio). Mean and maximal conduction times were calculated as the mean and the maximal conduction time measured throughout the eight MAP catheters for each pacing cycle length. All values given in milliseconds as mean ± standard deviation. Asterisks (*) mark significant differences between untreated and amiodarone-treated hearts. There was no significant difference in mean or maximal conduction times between groups, although there was a trend towards longer conduction times in amiodarone-treated hearts (p=0.07-0.14).

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Fig. 1
Fig. 2 A-B

ERC, BCL=400ms

ERC, BCL=600ms

Fig. 2 A-B
Figure 2 C-D

C. Sloped portion of RC, BCL=400ms

D. Sloped portion RC, BCL=600ms
Fig. 3

A

Probability of Arrhythmia Induction

![Bar chart showing the probability of arrhythmia induction at different stimulus strengths.](chart_A)

- **Base**
- **Amio**

Stimulus Strength (x diastolic threshold)

B

Proportion of Arrhythmia-Free Hearts

![Line graph showing the proportion of arrhythmia-free hearts.](chart_B)

- **Amio**

Stimulus Strength (x diastolic threshold)

C

Proportion of Arrhythmia

![Bar chart showing the proportion of arrhythmia at different conditions.](chart_C)

- **VF**
- **mVT**

Base

Amio

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Stimulus Strength (x diastolic threshold)

- Amio
- Base

**Fig. 5**