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Role of Repolarization Restitution in the Development of Coarse and Fine Atrial Fibrillation in the Isolated Canine Right Atria

ALEXANDER BURASHNIKOV, Ph.D. and CHARLES ANTZELEVITCH, Ph.D.

Masonic Medical Research Laboratory, Utica, New York, USA

Abstract

Introduction: Although the role of action potential duration restitution (APD-R) in the initiation and maintenance of ventricular fibrillation (VF) has been the subject of numerous investigations, its role in the generation of atrial fibrillation (AF) is less well studied. The cellular and ionic basis for coarse versus fine AF is not well delineated. **Methods and Results:** We measured APD-R during acetylcholine-mediated AF as well as during pacing (standard and dynamic protocols) in crista terminalis, pectinate muscle, superior vena cava, and appendage of isolated canine arterially perfused right atria (n = 15). Transmembrane action potential (TAP), pseudo-ECG, and isometric tension development were simultaneously recorded. Acetylcholine flattened APD-R measured by both standard and dynamic protocols, but promoted induction of AF. AF was initially *coarse*, converting to *fine* within 3–15 minutes of AF. Coarse, but not fine AF was associated with dramatic fluctuations in tension development, reflecting wide variations in intracellular calcium activity ($[Ca^{2+}]_i$). During coarse AF, APD-R data displayed a cloud-like distribution pattern, with a wide range of maximum APD-R slope (from 1.21 to 0.35). A maximum APD-R slope >1 was observed only in crista terminalis (3/10). The APD-R relationship was relatively linear and flat during fine AF. Reduction of $[Ca^{2+}]_i$ was associated with fine AF whereas augmentation of $[Ca^{2+}]_i$ with coarse AF. **Conclusions:** Our data indicate that while APD-R may have a limited role in the maintenance of coarse AF, it is unlikely to contribute to the maintenance of fine AF and that $[Ca^{2+}]_i$ dynamics determine the degree to which AF is coarse or fine.

Keywords

atrial fibrillation; action potential; restitution; coarse atrial fibrillation; fine atrial fibrillation

Introduction

The restitution hypothesis has been advanced as a mechanism for the occurrence of wavebreak during cardiac fibrillation.^{1,2} The restitution hypothesis maintains that the steepness of action potential duration restitution (APD-R) relationship largely determines the degree to which extent wavebreak occurs (the steeper the APD-R, the higher the probability of the wavebreak), and, as a consequence, initiation and maintenance of fibrillation. Flattening of APD-R using pharmacological agents has been proposed as an antifibrillatory strategy.^{2–4} While the role of APD-R in the initiation and maintenance of ventricular fibrillation (VF) has been the subject of numerous studies and vigorous debate,^{3,5–10} its role in the generation of atrial fibrillation (AF) is less well investigated. The cellular and ionic basis of coarse and fine AF has not been delineated. The present study examines the role of APD-R in the generation of coarse and fine

Address for correspondence: Dr. Alexander Burashnikov, Masonic Medical Research Laboratory, 2150 Bleecker Street, Utica, New York 13501. Fax: (315) 735 5648; E-mail: sasha@mmrl.edu.

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AF as well as the cellular and ionic mechanisms of these two types of AF using canine isolated coronary-perfused right atrial preparations.

Methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Arterially Perfused Canine Right Atrium (RA)

Dogs weighing 20–25 kg were anticoagulated with heparin and anesthetized with pentobarbital (30–35 mg/kg, i.v.). The chest was opened via a left thoracotomy, the heart excised, placed in a cardioplegic solution consisting of cold (4°C) Tyrode's solution containing 8.5 mM $[K^+]_0$ and transported to a dissection tray. Three-fourths of both ventricles were quickly removed. The ostium of the right coronary artery was cannulated with polyethylene tubing (i.d., 1.75 mm; o.d., 2.1 mm) and the preparation was perfused with cold Tyrode's solution (12–15°C) containing 8.5 $[K^+]_0$. With continuous coronary perfusion, all ventricular branches of the right coronary artery were immediately clamped with metal clips. The entire RA with a thin rim (<1 cm) of right ventricular tissue was carefully dissected from the remaining tissues and then the preparation was unfolded. Ventricular right coronary branches as well as the cut atrial branches were ligated using silk thread. The preparation was placed in a temperature-controlled bath (8 × 6 × 3 cm) and perfused at a rate of 8–10 mL/minute with Tyrode's solution (36.5 ± 0.5°C). The perfusate was delivered to the artery by a roller pump. An air trap was used to avoid bubbles in the perfusion line. The composition of the Tyrode's solution (in mM): NaCl 129, KCl 4, NaH₂PO₄ 0.9, NaHCO₃ 20, CaCl₂ 1.8, MgSO₄ 0.5, and D-glucose 5.5, buffered with 95% O₂ and 5% CO₂.

Transmembrane Action Potential (TAP)

TAP recordings were obtained using standard or floating glass microelectrodes (2.7 M KCl, 10–25 MΩ DC resistance) connected to a high input impedance amplification system (World Precision Instruments, FL). The signals were displayed on oscilloscopes, amplified, digitized and analyzed (Spike 2, Cambridge Electronic Design, Cambridge, England). TAPs were recorded from the endocardial surface of the crista terminalis (CT), pectinate muscle (PM), superior vena cava (SVC), and epicardial surface of the appendage (APG).

Electrocardiogram (Pseudo-ECG)

Pseudo-ECG was recorded using two electrodes consisting of AgCl half cells attached to Tyrode's-filled tapered polyethylene electrodes which were placed in the bath solution 1.0–1.2 cm from the opposite ends of the atrial preparation. AF was considered Coarse or Fine when the maximum amplitude of the ECG complexes was ≥80% or ≤60%, respectively, of that recorded during pacing at regular beats for each experiment.

Isometric Contractile Force

Isometric contractile force was recorded by attaching either intercaval band, APG, or SVC to a force-displacement transducer (Grass Instruments Co., Quincy, MA).

Experimental Protocols

Coronary-perfused spontaneously beating RA preparations were equilibrated in the tissue bath until electrically stable, usually 30 minutes. The preparations were then paced at a BCL of 500 ms, using a pair of thin silver electrodes insulated except at their tips (bipolar rectangular pulses of 2-ms duration and twice threshold intensity). Standard and dynamic restitution protocols were run first in control and then in the presence of acetylcholine (ACh, 0.5 μM).

Standard restitution protocols—Following a 10-beat drive train at a BCL of 500 ms (S_1 – S_1), a premature stimulus (S_2) at $2\times$ diastolic threshold was delivered at progressively shortened S_1 – S_2 coupling interval with a step of 100–5 ms until refractoriness was reached.

Dynamic restitution protocol—The atrial preparation was paced with a stepwise decrease in BCL (every 10 sec) starting from a BCL of 500 ms until atrial one to one capture failed. APD and DI were measured on the 10th second of pacing at each pacing rate. AF was induced by a single premature impulse or rapid pacing either after the completion of the pacing protocols or in the beginning of the experiments. During AF and pacing protocols, ECG, tension, and whenever possible up to three TAPs were simultaneously recorded for 40 minutes in each experiment. These three TAPs were simultaneously recorded from different atrial regions. In a separate set of experiments, the preparations were pretreated with sarcoplasmic reticulum (SR) calcium release blocker ryanodine (1.0 μ M) for 10 minutes with following addition of ACh (0.5 μ M) into the solution.

The APD-R curve was constructed by plotting APD₉₀ as a function of the preceding diastolic interval (DI₉₀). To facilitate comparison, all APD-R curves were fit to a single exponential, using SigmaPlot for Windows (SPSS Inc., Chicago, IL). Exceptions were fine AF APD-DI distributions, showing a flat functional relationship, which did not reasonably fit to any exponential function. One hundred consecutive beats of AF were processed and analyzed in each case. APD₉₀ and DI₉₀ measurement during AF were performed as follows: first the peak of phase 0 and the maximum resting potential of each action potential was determined, and APD₉₀ was obtained either from the 100% value of repolarization or depolarization (which was more negative). DI₉₀ was defined as the difference between the given CL and APD₉₀. The time moment of the maximum derivative of phase 0 was used for determination of CL. A negative DI value occurred when an APD₉₀ was longer than the CL. To minimize influence of electrotonic and graded responses, signals with APD₉₀ shorter than 20 ms as well as having $dV/dt \leq 5$ V/sec were disregarded.

Drugs

ACh and ryanodine (both dissolved in distilled water) were prepared fresh as a stock of 1 mM before each experiment.

Statistics

Statistical analysis was performed using a Student's *t*-test for unpaired data. All data are expressed as mean $\pm$ SD.

Results

ACh drastically reduced or eliminated contractility and increased APD dispersion, due to a greater APD shortening in PM, SVC, and APG, compared to CT (Table 1). ACh flattened the APD-R slopes measured using both standard and dynamic restitution protocols (Fig. 1, Table 1). The latter protocols always induced repolarization alternans in control (at a CL of ≤ 250 ms), but never in the presence of ACh. In the absence of ACh, only nonsustained AF (< 10 sec) could be induced in two of eight RAs despite a steep APD-R relationship. In contrast, AF was readily induced during exposure of the preparations to ACh despite a practically flat APD-R relationship (in 15/15 RA, with non-self-terminating AF in 9 RA). Sustained AF was coarse when first precipitated, gradually converting to fine AF within 3–15 minutes. To clearly distinguish between coarse and fine AF, we analyzed data recorded within 3 minutes (Coarse AF) and after 20 minutes (fine AF) of the start of AF. Coarse AF was associated with wide and highly irregular fluctuations of phasic tension (i.e., asynchronous contractility), indicating wide fluctuations of intracellular calcium activity ($[Ca^{2+}]_i$) (Fig. 2). Fine AF was characterized

by the absence of developed tension or small phasic tension, suggesting a relatively weak $[Ca^{2+}]_i$. This change of contractility during the course of AF was similar irrespective of the location of the tension recording (intercaval band, APG, or SVC). Highly irregular and relatively regular APDs and DI were recorded during coarse and fine AF, respectively (Fig. 3). Average CL, APD₉₀, and DI₉₀ during coarse and fine AF recorded from the same RA are shown in Figure 4. Similar results were obtained in eight other experiments. During coarse AF, DI-APD data displayed a cloud-like pattern, yielding a poor functional relationship ($r^2 = 0.21-0.46$) and a wide range of maximum slopes in CT ($0.46-1.21$, 0.83 ± 0.24 , $n = 10$, $P < 0.05$ vs all other regions), PM ($0.41-0.76$, 0.60 ± 0.17 , $n = 5$), SVC ($0.42-0.71$, 0.57 ± 0.20 , $n = 4$), and APG ($0.35-0.63$, 0.49 ± 0.14 , $n = 5$) (Fig. 3). Note that a maximum slope of APD-R > 1 was observed only in CT (in 3/10 cases). During fine AF, a practically flat APD-R relationship was recorded in 100% of cases in the four atrial regions ($n = 5$ for each) (Fig. 3).

To further investigate the relationship between the dynamics of $[Ca^{2+}]_i$ and the coarseness of AF, we examined the effects of reduction and augmentation of $[Ca^{2+}]_i$ on the electrical and mechanical parameters during AF. In the presence of the SR calcium release blocker, ryanodine ($1.0 \mu M$) and ACh ($0.5 \mu M$), AF was induced in three of seven RAs (persistent AF in 2 and nonsustained in 1). These AF episodes were fine from the start, failing to show robust fluctuations of phasic tension and displaying a flat or stationary DI-APD relationship (Fig. 5). In contrast, augmentation of $[Ca^{2+}]_i$ during fine AF (achieved by removal of ACh from the coronary perfusate¹¹) always (8/8 episodes in 5 atria) produced an amplification or appearance of tension development with fluctuation of phasic tension, which was invariably associated with the conversion to coarse AF (Fig. 6). These results suggest a critical role for $[Ca^{2+}]_i$ in the manifestation of coarse AF. APD prolongation attending removal of ACh prolongs the reentrant wavelength and may thus contribute to conversion of fine to coarse AF.

Discussion

Our results suggest that (1) atrial APD restitution properties may have a limited role in the maintenance of coarse AF, but are unlikely to be a determining factor in the maintenance of fine AF; and (2) the coarseness of AF is importantly modulated by $[Ca^{2+}]_i$.

Coarse and fine AFs were defined based on the surface ECG as having relatively large and small amplitude ECG atrial deflections, respectively.¹² Wells et al.¹³ using bipolar electrodes and Konings et al.¹⁴ applying multielectrode mapping techniques described four and three types of AF, respectively, in human. In general, when going from type I to type III, AF was associated with an increasing complexity of surface activation, shortening of local CL, and slowing of conduction velocity. Coarse AF is best represented by types I and II of AF and fine AF by type III and IV.¹³ To the best of our knowledge, there are no data on the cellular and ionic basis for coarse versus fine AF. Our study demonstrates that persistent AF leads to abbreviation of APD, prolongation of DI, and progressive loss of phasic contraction, which is associated with conversion of coarse AF to fine AF. Likewise, block of SR calcium release with ryanodine leads to loss of phasic contractions and to induction of only fine AF. Moreover, restoration of phasic contraction by removal of ACh, leads to conversion of fine to coarse AF. Thus, the degree to which AF is electrically coarse appears to depend on the degree of $[Ca^{2+}]_i$.

Two major hypotheses have been advanced to account for the maintenance of VF and AF: the multiple wavelet and single source hypotheses.^{15,16} The multiple wavelet theory proposes continuous wavebreak as the engine of VF/AF, whereas the single source theory suggests that continuous wavebreak is a consequence of a rapidly activating relatively stable source. There is experimental evidence in support of both theories.^{16,17} The restitution hypothesis has been advanced as a mechanism for the occurrence of wave breakup.^{1,2} According to the restitution

hypothesis, the relation between APD and the previous DI largely determines the probability of wave breakup at restitution slopes ≥ 1 . It is well appreciated however, that in the real heart, apart from the APD-R, a number of other factors are likely to contribute to the wave breakup during VF/AF (such as cardiac memory, conduction abnormalities, intracellular calcium cycling, ischemia, anisotropy, anatomical obstacles, etc.),^{7,8,10,18} so that wave breakup can occur when the APD-R slope is less than 1 and fail to occur when the slope is more than 1.^{8,18} These modulating factors notwithstanding, according to the restitution hypothesis, a steepening of DI-APD relation should promote and a flattening of DI-APD relation should prevent/terminate AF/VF.

While the role of APD-R in the initiation and maintenance of VF has been the subject of numerous studies and debates,^{3,5-10} its contribution to the generation of AF is much less studied. A major criticism of the application of the restitution hypothesis to the AF is that the atria susceptible to AF (due to electrical remodeling or vagal influences) are associated with loss of refractoriness and APD rate-adaptation as well as fluttering of the APD-R curve,¹⁹⁻²² including protocols at which pacing rates were similar to those encountered during AF.²¹ Apparent exceptions to this rule include the AF related to congestive heart failure²³ or chronic ventricular myocardial infarction.²⁴ In our study, cholinergic influences flattened APD-R slope, but promoted AF, indicating that the APD-R does not play a role in the initiation of cholinergically mediated AF. However, the presence of pronounced temporal APD and DI variability in the early stages of AF (i.e., coarse AF), gives rise to a wide range of APD-R maximum slopes (0.35–1.21, with a slope > 1 recorded only in CT), raising the possibility that APD-R in the region of CT may facilitate wave-break and thus play a limited role in maintenance of coarse AF. In contrast, the maintenance of fine AF was invariably associated with little to no temporal APD variability and with flat APD-R relationships, suggesting that APD-R is not involved in the maintenance of the established stage of AF. Interestingly, results of a multielectrode mapping study in canine isolated perfused RA indicate that the initial phase of ACh-mediated AF is caused by multiple wavelets, and the established phase of AF by a single stable rotor, associated with fibrillatory conduction.²⁵ Thus, coarse AF (initial stages) and fine AF (established stages) may have different underlying mechanisms (compare their quite different global and local electrical activities; Figs. 2 and 3)

Intrinsic APD heterogeneity and natural anatomic obstacles contribute importantly to wavebreak and AF generation. It has been shown that the canine right atrial PM network is capable of producing wave break and reentry.²⁶ Canine RA displays intrinsic endocardial, epicardial, and transmural repolarization heterogeneities under baseline conditions,²⁷ which are known to be accentuated by cholinergic influences,^{28,29} similar to that demonstrated in the present study. Of note, ventricular transmural repolarization heterogeneity is known to be involved in the generation of arrhythmias in a variety of syndromes, including the long and short QT syndromes, Brugada syndrome.³⁰ The apparent spatial dispersion of repolarization observed during coarse AF (between CT and adjacent regions; Fig. 4) may be important for the maintenance of coarse AF. This hypothesis is consistent with that of Moe et al. proposing that intrinsic electrical heterogeneity is the basis for AF maintenance.¹⁵

The appearance of a dramatic temporal APD heterogeneity (and, as a result, steep APD-R slopes) at the start of AF is likely to be related to AF-induced $[Ca^{2+}]_i$ fluctuations associated with intracellular calcium loading.^{31,32} SR calcium release is known to significantly affect APD.³³ By the same token, the flat APD-R relationship and absence of temporal APD dispersion during fine AF may be explained by lack of $[Ca^{2+}]_i$ fluctuations. The absence of temporal APD heterogeneity and contractility during AF in the presence of ryanodine provides support for this hypothesis. The presence of a wide range of APD-R slopes during coarse AF may be explained by difference in $[Ca^{2+}]_i$ dynamics. Dynamic changes in $[Ca^{2+}]_i$ can influence APD by modulating any one of a number ion channel currents and exchangers, prominent

among them are the slowly activating delayed rectifier, IKs, and the sodium-calcium exchanger, INa-Ca.

It has been suggested that conduction disturbance may account for VF/AF, in cases of flat APD-R relationship.^{34,35} ACh does not affect conduction in the canine atrium,³⁶ arguing against the participation of this mechanism in the early stage of ACh-mediated AF. Conduction slowing may help to sustain AF in later stages. Calcium loading, induced by AF, is known to reduce cellular coupling, which impairs conduction, thus sustaining AF. It is noteworthy that partial cellular uncoupling with heptanol promotes AF vulnerability, without changing APD-R properties in isolated canine perfused RA.³⁵

Clinical Implications

Our results indicate that pharmacological modification of the APD-restitution relationship is unlikely to be effective in the treatment of fine AF, the most prevalent form of clinical AF. Knowledge of the role of intracellular calcium dynamics in the generation of coarse versus fine AF, may help in the development of new therapeutic strategies for the treatment of AF or thromboembolic complications secondary to AF, since fibrillatory wave amplitude has been shown to be a predictor of thromboembolic risk in some patients.³⁷

Study Limitations

Extrapolations of the results of the present study to the clinic should be done with a caution. Our results were obtained in “normal” atria, whereas most clinical AF occurs in electrically remodeled or structurally compromised atria. Some forms of paroxysmal and lone AF, however, occur in the absence of any electrical or structural abnormalities. We used ACh to mimic electrically remodeled atria as well as parasympathetic influences. The degree to which vagotonic-mediated mechanisms contribute to the development of AF in the clinic is not well established. Recent studies have demonstrated that ablation of parasympathetic ganglia can successfully control some forms of AF.³⁸ It is noteworthy that ACh-mediated electrical changes (such as APD abbreviation and fluttering APD-R) have also been reported in electrically remodeled animal atria²⁰ as well as in the atria of the humans susceptible to AF,¹⁹ suggesting that the results obtained in the present study may have relevance to a wide spectrum of AF encountered in the clinic.

The standard and dynamic APD-R protocols, used in our study, are convenient, but not adequate for the full estimation of cardiac restitution properties relevant to cardiac fibrillation.^{8,18} A recently introduced “restitution portrait” pacing protocol,³⁹ is more complex, but may give a better estimation of the cardiac APD-R. It is noteworthy that atrial APD rate adaptation, an important pillar of “restitution portrait,” is small or absent in the presence of ACh.

The maximum slope of APD-R measured during AF/VF provides an index of the role of restitution in AF. APD-R slope value is affected by a number of factors, including: (1) the method of measurement of APD/DI (APD₉₀, APD₇₀, constant voltage, etc); (2) whether one includes or excludes electrotonus/graded responses/mechanical artifacts and/or negative DI values; and (3) the fitting function selected (exponential, sigmoidal, etc.). However, these limitations should not significantly affect the interpretation of our results, since we describe a *qualitative* difference between coarse and fine AF, that is, the presence or absence of temporal APD heterogeneity, respectively. Similarly, our conclusions regarding the role of $[Ca^{2+}]_i$ in AF are based on distinct *qualitative* differences in contractility observed during coarse and fine AF.

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References

1. Karma A. Electrical alternans and spiral wave breakup in cardiac tissue. *Chaos* 1994;4:461–472. [PubMed: 12780121]
2. Weiss JN, Garfinkel A, Karagueuzian HS, Qu Z, Chen PS. Chaos and the transition to ventricular fibrillation: a new approach to antiarrhythmic drug evaluation. *Circulation* 1999;99:2819–2826. [PubMed: 10351978]
3. Garfinkel A, Kim YH, Voroshilovsky O, Qu Z, Kil JR, Lee MH, Karagueuzian HS, Weiss JN, Chen PS. Preventing ventricular fibrillation by flattening cardiac restitution. *Proc Natl Acad Sci USA* 2000;97:6061–6066. [PubMed: 10811880]
4. Xie F, Qu Z, Garfinkel A, Weiss JN. Electrical refractory period restitution and spiral wave reentry in simulated cardiac tissue. *Am J Physiol Heart Circ Physiol* 2002;283:H448–H460. [PubMed: 12063320]
5. Laurita KR, Girouard SD, Rosenbaum DS. Modulation of ventricular repolarization by a premature stimulus. Role of epicardial dispersion of repolarization kinetics demonstrated by optical mapping of the intact guinea pig heart. *Circ Res* 1996;79:493–503. [PubMed: 8781482]
6. Koller ML, Riccio ML, Gilmour RF Jr. Dynamic restitution of action potential duration during electrical alternans and ventricular fibrillation. *Am J Physiol* 1998;275:H1635–H1642. [PubMed: 9815071]
7. Banville I, Gray RA. Effect of action potential duration and conduction velocity restitution and their spatial dispersion on alternans and the stability of arrhythmias. *J Cardiovasc Electrophysiol* 2002;13:1141–1149. [PubMed: 12475106]
8. Ideker RE, Rogers JM, Gray RA. Steepness of the restitution curve: A slippery slope? *J Cardiovasc Electrophysiol* 2002;13:1173–1175. [PubMed: 12475112]
9. Taggart P, Sutton P, Chalabi Z, Boyett MR, Simon R, Elliott D, Gill JS. Effect of adrenergic stimulation on action potential duration restitution in humans. *Circulation* 2003;107:285–289. [PubMed: 12538429]
10. Banville I, Chattipakorn N, Gray RA. Restitution dynamics during pacing and arrhythmias in isolated pig hearts. *J Cardiovasc Electrophysiol* 2004;15:455–463. [PubMed: 15089996]
11. Wang YG, Huser J, Blatter LA, Lipsius SL. Withdrawal of acetylcholine elicits Ca²⁺-induced delayed afterdepolarizations in cat atrial myocytes. *Circulation* 1997;96:1275–1281. [PubMed: 9286959]
12. Hewlett AW, Wilson FN. Coarse auricular fibrillation in man. *Arch Intern Med* 1915;15:788–794.
13. Wells JL Jr, Karp RB, Kouchoukos NT, MacLean WA, James TN, Waldo AL. Characterization of atrial fibrillation in man: Studies following open heart surgery. *PACE* 1978;1:426–438. [PubMed: 95635]
14. Konings KT, Kirchhof CJ, Smeets JR, Wellens HJ, Penn OC, Allessie MA. High-density mapping of electrically induced atrial fibrillation in humans. *Circulation* 1994;89:1665–1680. [PubMed: 8149534]
15. Moe GK, Rheinboldt WC, Abildskov JA. A computer model of atrial fibrillation. *Am Heart J* 1964;67:200–220. [PubMed: 14118488]
16. Scherf D, Romano FJ, Terranova R. Experimental studies on auricular flutter and auricular fibrillation. *Am Heart J* 1948;36:241–251.
17. Allessie MA, Lammers WJEP, Bonke FIM, Hollen J. Experimental evaluation of Moe's multiple wavelet hypothesis of atrial fibrillation. In: Zipes DP, Jalife J, editors. *Cardiac Electrophysiology and Arrhythmias*. Grune & Stratton; 1985.
18. Fenton FH, Cherry EM, Hastings HM, Evans SJ. Multiple mechanisms of spiral wave breakup in a model of cardiac electrical activity. *Chaos* 2002;12:852–892. [PubMed: 12779613]
19. Attuel P, Childers R, Cauchemez B, Poveda J, Mugica J, Coumel P. Failure in the rate adaptation of the atrial refractory period: Its relationship to vulnerability. *Int J Cardiol* 1982;2:179–197. [PubMed: 6185445]

20. Wijffels MC, Kirchhof CJ, Dorland R, Allessie MA. Atrial fibrillation begets atrial fibrillation. A study in awake chronically instrumented goats. *Circulation* 1995;92:1954–1968. [PubMed: 7671380]
21. Workman AJ, Kane KA, Rankin AC. The contribution of ionic currents to changes in refractoriness of human atrial myocytes associated with chronic atrial fibrillation. *Cardiovasc Res* 2001;52:226–235. [PubMed: 11684070]
22. Hara M, Shvilkin A, Rosen MR, Danilo P Jr, Boyden PA. Steady-state and nonsteady-state action potentials in fibrillating canine atrium: Abnormal rate adaptation and its possible mechanisms. *Cardiovasc Res* 1999;42:455–469. [PubMed: 10533581]
23. Li D, Fareh S, Leung TK, Nattel S. Promotion of atrial fibrillation by heart failure in dogs: Atrial remodeling of a different sort. *Circulation* 1999;100:87–95. [PubMed: 10393686]
24. Miyauchi Y, Zhou S, Okuyama Y, Miyauchi M, Hayashi H, Hamabe A, Fishbein MC, Mandel WJ, Chen LS, Chen PS, Karagueuzian HS. Altered atrial electrical restitution and heterogeneous sympathetic hyper-innervation in hearts with chronic left ventricular myocardial infarction: Implications for atrial fibrillation. *Circulation* 2003;108:360–366. [PubMed: 12835207]
25. Schuessler RB, Grayson TM, Bromberg BI, Cox JL, Boineau JP. Cholinergically mediated tachyarrhythmias induced by a single extrastimulus in the isolated canine right atrium. *Circ Res* 1992;71:1254–1267. [PubMed: 1394883]
26. Wu TJ, Yashima M, Xie F, Athill CA, Kim YH, Fishbein MC, Qu Z, Garfinkel A, Weiss JN, Karagueuzian HS, Chen PS. Role of pectinate muscle bundles in the generation and maintenance of intra-atrial reentry: Potential implications for the mechanism of conversion between atrial fibrillation and atrial flutter. *Circ Res* 1998;83:448–462. [PubMed: 9721702]
27. Burashnikov A, Mannava S, Antzelevitch C. Transmembrane action potential heterogeneity in the canine isolated arterially-perfused atrium: Effect of IKr and Ito/IKur block. *Am J Physiol* 2004;286:H2393–H2400.
28. Zipes DP, Mihalick MJ, Robbins GT. Effects of selective vagal and stellate ganglion stimulation of atrial refractoriness. *Cardiovasc Res* 1974;8:647–655. [PubMed: 4434368]
29. Burashnikov A, Antzelevitch C. Heterogeneous distribution of action potential duration in canine isolated perfused right atrium and its accentuation of acetylcholine. *Pacing Clin Electrophysiol* 2000;23:II-729.(abstract)
30. Antzelevitch, C.; Burashnikov, A.; Di Diego, JM. Cellular and ionic mechanisms underlying arrhythmogenesis. In: Gussak, I.; Antzelevitch, C., editors. *Cardiac Repolarization. Bridging Basic and Clinical Sciences*. Humana Press; Totowa, NJ: 2003.
31. Leistad E, Christensen G, Ilebekk A. Atrial contractile performance after cessation of atrial fibrillation. *Am J Physiol* 1993;264:H104–H109. [PubMed: 8430837]
32. Burashnikov A, Antzelevitch C. Reinduction of atrial fibrillation immediately after termination of the arrhythmia is mediated by late phase 3 early afterdepolarization-induced triggered activity. *Circulation* 2003;107:2355–2360. [PubMed: 12695296]
33. Pruvot EJ, Katra RP, Rosenbaum DS, Laurita KR. Role of calcium cycling versus restitution in the mechanism of repolarization alternans. *Circ Res* 2004;94:1083–1090. [PubMed: 15016735]
34. Chen PS, Wu TJ, Ting CT, Karagueuzian HS, Garfinkel A, Lin SF, Weiss JN. A tale of two fibrillations. *Circulation* 2003;108:2298–2303. [PubMed: 14609997]
35. Ohara T, Qu Z, Lee MH, Ohara K, Omichi C, Mandel WJ, Chen PS, Karagueuzian HS. Increased vulnerability to inducible atrial fibrillation caused by partial cellular uncoupling with heptanol. *Am J Physiol Heart Circ Physiol* 2002;283:H1116–H1122. [PubMed: 12181142]
36. Schuessler RB, Bromberg BI, Boineau JP. Effect of neurotransmitters on the activation sequence of the isolated atrium. *Am J Physiol* 1990;258:H1632–H1641. [PubMed: 1972866]
37. Mutlu B, Karabulut M, Eroglu E, Tigen K, Bayrak F, Fotbolcu H, Basaran Y. Fibrillatory wave amplitude as a marker of left atrial and left atrial appendage function, and a predictor of thromboembolic risk in patients with rheumatic mitral stenosis. *Int J Cardiol* 2003;91:179–186. [PubMed: 14559128]
38. Pappone C, Santinelli V, Manguso F, Vicedomini G, Gugliotta F, Augello G, Mazzone P, Tortoriello V, Landoni G, Zangrillo A, Lang C, Tomita T, Mesas C, Mastella E, Alfieri O. Pulmonary vein denervation enhances long-term benefit after circumferential ablation for paroxysmal atrial fibrillation. *Circulation* 2004;109:327–334. [PubMed: 14707026]

39. Kalb SS, Dobrovolny HM, Tolkacheva EG, Idriss SF, Krassowska W, Gauthier DJ. The restitution portrait: a new method for investigating rate-dependent restitution. *J Cardiovasc Electrophysiol* 2004;15:698–709. [PubMed: 15175067]

Figure 1.

Acetylcholine reduces slopes of the APD-R relationships. A-B: superimposed action potentials recorded during standard restitution protocols under control conditions and in the presence of ACh from CT. B-C: typical plots of DI-APD relations obtained by standard (C) and dynamic (D) restitution protocols in the absence and presence of ACh. Due to repolarization alternans at $CL \leq 250$ ms, the dynamic restitution plot recorded under control conditions is constructed from alternating short-long diastolic intervals and APDs, as described in the original paper dealing with the dynamic restitution protocol.⁶

Figure 2.

Tension development during coarse and fine AF. Coarse AF is associated with large and fine AF with small developed tension fluctuations, suggesting prominent and weak fluctuations in $[Ca^{+2}]_i$, respectively.

Figure 3.
Action potential recordings obtained during coarse and fine AF as well as respective APD-R relations. The action potentials were recorded from CT.

Figure 4.

Average APD₉₀, DI₉₀, and CL during coarse and fine AF in CT, PM, SVC, and APG. Data were obtained from the same RA (n = 100 consecutive beats for each). * P < 0.05 versus coarse AF. †, §P < 0.05 versus same parameter in the CT region during coarse AF. In all regions average APD is shorter and DI is longer during fine AF, compared to coarse AF. Average CL is shorter during fine versus coarse AF only in CT.

Figure 5. Effect of reduction in $[Ca^{2+}]_i$ on electrical and mechanical characteristics of AF. A: tension, ECG, and action potential tracings simultaneously recorded during second minute of AF induced in the presence of ryanodine and ACh. B: a plot of APD-R relationship obtained under these conditions.

Figure 6. Effect of augmentation of $[Ca^{2+}]_i$ on electrical and mechanical parameters during AF. Shown are developed tension and ECG tracings simultaneously recorded during fine AF (16 min after start of AF). ACh was removed from the coronary perfusate at the arrow, leading to the development of irregular phasic contractions and coarse AF.

TABLE 1
Effects of ACh on APD₉₀ and the maximum slope of APD-R

	APD ₉₀ (ms)		Slope _{max} (standard)		Slope _{max} (dynamic)	
	Control	ACh	Control	ACh	Control	ACh
CT	196 ± 17	85 ± 14 [*]	0.54 ± 0.16	0.18 ± 0.05 [*]	4.79 ± 0.83	0.36 ± 0.10 [*]
PM	178 ± 12 [†]	50 ± 9 ^{*‡}	0.57 ± 0.10	0.14 ± 0.06 [*]	3.81 ± 0.81	0.24 ± 0.05 [*]
SVC	184 ± 11 [†]	53 ± 10 ^{*‡}	0.59 ± 0.18	0.15 ± 0.07 [*]	4.20 ± 0.67	0.27 ± 0.08 [*]
APG	180 ± 8 [†]	48 ± 7 ^{*‡}	0.52 ± 0.13	0.14 ± 0.06 [*]	4.06 ± 0.74	0.25 ± 0.11 [*]

The maximum slope of APD-R (Slope_{max}) was determined by standard and dynamic APD-R pacing protocols.

^{*} P < 0.001 versus respective control

[†] P < 0.05 versus CT in control

[‡] P < 0.001 versus CT in the presence of ACh

[§] P < 0.05 versus CT in the presence of ACh. n = 4–10.