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Cellular Basis for Atrial Fibrillation in an Experimental Model of Short QT1: Implications for a Pharmacologic Approach to Therapy

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Abstract

Background—Short QT syndrome (SQT) 1 is an inherited sudden death syndrome often associated with atrial fibrillation (AF). We examined the cellular basis for AF in a newly developed experimental atrial model of SQT1.

Methods—Action potentials (AP) were recorded from pectinate muscle (PM) and crista terminalis (CT) regions of coronary-perfused canine right atrial preparations, together with a pseudo-ECG. The I_{K_r} agonist PD-118057 (20 μ M) was used to mimic the gain of function in I_{K_r} known to underlie SQT1.

Results—The I_{K_r} agonist significantly abbreviated AP duration (APD) of CT and PM and effective refractory period (ERP) measured in PM ($n = 28$). Spatial dispersion of repolarization (SDR), defined as inter-regional differences of APD, increased from 27 ± 17 to 51 ± 32 ($p = 0.002$; $n = 28$). AF could be induced by a single premature stimulus after, but not before exposure to PD-118057, in 26/28 (93%) preparations. Quinidine (10 μ M), which prolonged APD and ERP, but not lidocaine (20 μ M) or E-4031 (5 μ M), which prolonged only ERP or APD, respectively, was effective in preventing the PD-118057-mediated AF. In the presence of PD-118057, isoproterenol (100 nM) further abbreviated both APD and ERP and facilitated induction of sustained AF in 5/6 preparations.

Conclusions—The I_{K_r} agonist recapitulates the electrophysiologic and arrhythmic manifestations of SQT1. Abbreviation of APD, ERP and amplification of SDR predispose to the development of AF by creating the substrate for reentry. Quinidine, but not E-4031 or lidocaine, was effective in preventing AF in this setting.

Keywords

Arrhythmia; Electrophysiology; Antiarrhythmic drugs; Quinidine; Isoproterenol

Introduction

The short QT syndrome (SQTS) is an inherited sudden cardiac death syndrome characterized by abnormally short QTc intervals (<360 ms) ¹ and an increased propensity for development of atrial and ventricular tachyarrhythmias.^{2–6} Although a short QT interval is the hallmark of

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Conflict of Interest: None

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SQTS, these patients tend to have other distinguishing electrocardiogram (ECG) characteristics, including tall peaked, symmetrical or asymmetrical T waves and occasional depression of the PR interval.⁶ In the largest series available (n= 29),⁷ 31% of patients presented with atrial fibrillation (AF) or flutter.

Thus far, mutations in 5 different genes encoding cardiac ion channels have been identified in familial or sporadic AF cases. Gain-of-function mutations in *KCNH2*⁷, *KCNQ1*⁸ and *KCNJ2*⁹ genes encoding for the rapidly and slowly activating potassium channel currents (I_{Kr} and I_{Ks}) and the inward rectifier potassium channel current (I_{K1}), give rise to the SQT1, SQT2 and SQT3 forms of SQTS. Our group was the first to identify a missense mutation (N588K) in *KCNH2* in 2 unrelated families.⁷ This mutation has proved to be a hotspot for SQTS. Analysis of the current generated following transfection of the mutated channel into human embryonic kidney (HEK) cells showed that the mutation shifted inactivation of the *KCNH2* channels out of the range of the action potential, thus producing a large gain of function in I_{Kr} . The gain of function in I_{Kr} is responsible for abbreviation of the action potential duration (APD).

A heterogeneous abbreviation of APD and refractoriness in different regions of ventricular myocardium is believed to create the substrate for reentrant arrhythmias within the ventricle. Evidence in support of this hypothesis in SQTS derives from two models involving the left ventricular coronary-perfused wedge preparations exposed to the K channel opener pinacidil¹⁰ or to the I_{Kr} agonist, PD-118057.¹¹ It is noteworthy that the same point mutation in *KCNH2* shown to be associated with sudden cardiac death (SCD), was reported by Hong and co-workers¹² to be associated exclusively with AF.

Pharmacotherapy of SQTS would appear to be fairly straightforward since many of the traditional antiarrhythmics can act to prolong APD via inhibition of I_{Kr} and thus antagonize the increase in net depolarizing current produced by the genetic defects. However, this logical approach proved to be less than straightforward when first implemented in the clinic. Attempts to prolong the QT interval using the selective I_{Kr} blocker, d-sotalol, in SQT1 patients met with failure.¹³ Subsequent studies identified quinidine as an effective agent for both ventricular fibrillation (VF) and AF associated with SQT1,^{14,13} suggesting a common mechanism. To our knowledge there are no experimental models of SQT syndrome in the atria and little in the way of experimental data that are helpful in advancing our understanding of the mechanism (s) underlying arrhythmogenesis in this syndrome or the approach to therapy of AF in these patients.

Methods

Arterially-perfused whole canine right atrium (RA)

Mongrel dogs (≥ 1 year old) 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 $[K^+]_o$ and transported to a dissection tray. The ostium of the right coronary artery was cannulated and the preparation was perfused with cold Tyrode's solution (12–15°C) containing 8.5 $[K^+]_o$. The RA preparation (Fig. 1) was placed in a temperature-controlled bath, maintained at 37°C throughout all experiments and perfused and superfused at a rate of 5–10 and 8–10 ml/min, respectively, with Tyrode's solution. The composition of the Tyrode's solution was as follows (in mmol/L): NaCl 129, KCl 4, NaH_2PO_4 0.9, $NaHCO_3$ 20, $CaCl_2$ 1.8, $MgSO_4$ 0.5, D-glucose 5.5 (pH=7.4). Transmembrane action potential (AP) recordings were obtained either differentially or referenced to ground using standard and floating glass microelectrodes (2.7 M KCl, 10–25 M Ω DC resistance) connected to a high input impedance amplification system (WPI, Sarasota, FL). The signals

were displayed on Tektronix oscilloscopes, amplified (model 1903–4 programmable amplifiers, Cambridge Electronic Design (CED, Philadelphia, PA), digitized (model 1401 AD/DA system, CED), analyzed (Spike 2 for Window) and stored on computer hard drive. A pseudo-ECG was recorded with 2 electrodes consisting of Ag/AgCl half cells were placed in the Tyrode's solution bathing the preparation, 1.0 to 1.2 cm from opposite sides of the atrial or to the ventricular coronary-perfused preparation. The basic stimulus (S_1) was delivered at progressively endocardium at a BCL of 500 ms. A premature stimulus (S_2) was delivered briefer S_1 – S_2 intervals (with 10 ms resolution; stimulation with a diastolic threshold of excitation X 2 amplitude) until the refractory period was reached in an attempt to induce AF. The effective refractory period (ERP) was defined as the longest S_1 – S_2 interval at which S_2 failed to capture. AF inducibility was examined under each condition separately (control, PD-118057 + Quinidine, + Lidocaine, + E-4301 or + Isoproterenol). APD dispersion was determined as the inter-regional atrial differences between the longest and shortest APD measured at 70% and 90% repolarization (APD₇₀ and APD₉₀, respectively), recording from the crista terminalis (CT) and the pectinate muscle (PM).

The dominant frequency (DF) of activation during AF was determined using Fast Fourier transform (FFT) (3.93 sec duration, 8192 bins, providing 0.254-Hz spectral resolution, Spike 2 for Windows, Cambridge, England).

Drugs

PD-118057, a kind gift of Pfizer Pharmaceuticals, was prepared as a 10 mM stock in 100% Dimethyl Sulfoxide (DMSO) and added to Tyrode's solution at final concentration of 20 μ M. Quinidine, Lidocaine and E-4301 were prepared as a 20 mM stock in Millipore water and added to Tyrode's solution to achieve final concentrations of 10 μ M, 20 μ M and 5 μ M, respectively. Isoproterenol was prepared as a stock solution of 1 mM in Millipore water and added to Tyrode's solution to achieve final concentrations of 100 nM.

Statistical Analysis

Statistical analysis was performed through the use of a paired or unpaired Student's t test and one way repeated-measures or multiple comparisons ANOVA followed by Bonferroni's test, as appropriate. All data are expressed as mean \pm standard deviation (SD). Statistical significance was assumed at $P < 0.05$.

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written. All experiments were performed in conformance with the guidelines of the Institutional Animal Care Committee.

Results

The I_{K_r} agonist PD-118057 abbreviated APD₉₀ from 236 ± 23 to 195 ± 30 ms and APD₇₀ from 164 ± 21 to 116 ± 30 ms in CT ($p < 0.001$; $n = 28$) and APD₉₀ from 212 ± 20 to 139 ± 3 and APD₇₀ from 142 ± 19 to 67 ± 25 in PM ($p < 0.001$; $n = 28$) (Fig 2). Abbreviation of APD was accompanied by abbreviation of ERP in PM from 149 ± 12 to 100 ± 10 ($p < 0.001$; $n = 28$) (Figs. 2 and 3). However, PD-118057 produced a greater abbreviation of APD₇₀ in PM than in CT (Fig. 2). This differential abbreviation of APD in PM and CT, significantly increased the spatial dispersion of repolarization (SDR) from 27 ± 17 to 51 ± 32 ($p = 0.002$; $n = 28$) (Fig. 3).

Under these conditions, with basic stimulation (S_1) at a CL of 500 ms, we applied single premature stimuli (S_2) to PM endocardium at progressively shorter S_1 – S_2 intervals in an attempt to induce AF. A single extrastimulus applied to the PM region gave rise to non-sustained (< 30 s) AF in 26/28 (93%) preparations exposed to PD-118057 (Fig. 4). In only one

of these, did non-sustained AF degenerate into sustained AF. In one preparation, permanent AF was induced. AF (>4 beats) could not be induced in any preparation under control conditions. Thus, PD-118057 via its actions to increase I_{Kr} , markedly abbreviated APD and increased SDR, leading to the development of AF.

We next examined the effectiveness of various antiarrhythmic drugs to suppress AF in the model. We selected drugs representing Vaughan Williams Class IA (quinidine), Class IB (lidocaine) and Class III (the I_{Kr} blocker E-4301). The effects of these agents were evaluated after induction of AF using PD-118057. Each agent was added to the coronary perfusate in the continued presence of PD-118057. Table 1 summarizes the effect of these drugs on APD₇₀ in PM and CT, ERP and AF inducibility.

Lidocaine, a selective sodium channel current (I_{Na}) blocker, was effective in reversing the I_{Kr} agonist-induced abbreviation of ERP, but not APD, whereas E-4031, a selective I_{Kr} blocker, reversed both the PD-118057-induced abbreviation of APD and ERP, but not to control levels. Quinidine, possessing both I_{Kr} and I_{Na} inhibitory effects, similarly reversed both the APD and ERP abbreviation, and was the only agent effective in preventing the induction of AF in all cases (Fig. 5). Lidocaine, despite restoring ERP to control values, permitted induction of brief episodes of atrial tachycardia in 4/5 preparations (Fig. 6) and AF in 1/5.

Addition of isoproterenol (100 nM) to the coronary-perfusate containing PD-118057 further abbreviated ERP and APD₇₀ in both CT and PM (Table 1 and Fig. 5D). With PD-118057 alone, a premature stimulus was effective in inducing non-sustained AF in 6/6 preparations. After adding isoproterenol, a premature stimulus induced sustained AF in 5/6 preparations (Fig. 7). The dominant frequency of atrial activation was much faster after isoproterenol (12 ± 1 vs. 8 ± 0.8 Hz; $p = 0.002$). In the absence of PD-118057, isoproterenol alone did not induce sustained AF in 8/8 preparations, although non-sustained AF (<30 sec) could be induced in 2/8 preparations.

Discussion

To our knowledge, this is the first experimental model of SQT syndrome involving atria. Using PD-118057, an I_{Kr} agonist, we were able to recapitulate the clinical atrial arrhythmic manifestations of SQT1 and examine the cellular mechanisms underlying the development of AF as well as the mechanism of action of antiarrhythmic drug therapy for AF under SQT1 conditions. The model also enabled us to test the hypothesis that sympathetic stimulation may contribute to or aggravate the atrial arrhythmic burden in patients with shorter than normal QT intervals.

Although AF is the most common arrhythmia in humans, our knowledge about the ionic mechanisms underlying this arrhythmia is far from complete. Genetic syndromes and ion channel mutations can provide insight into our understanding of the basic mechanisms contributing to the development of AF. In our SQT1 model, abbreviation of APD facilitated the development of the substrate for reentry by abbreviating ERP and reducing wavelength as well as by amplifying SDR, secondary to heterogeneous abbreviation of APD. Interestingly, patients with lone AF were found in one study¹⁵ to have shorter QT intervals compared to control. These findings demonstrated that even in the absence of genetically identifiable channelopathies, patients with AF and no structural heart disease have significantly shorter QT intervals than their age- and gender-matched healthy counterparts.¹⁵

Human atrial cells isolated from patients with AF have been found to have reduced levels of L-type calcium current ($I_{Ca,L}$) and transient outward current (I_{to}) and increased levels of inward rectifier potassium current (I_{K1}) and acetylcholine-activated potassium current (I_{K-ACh}).¹⁶ Activation of I_{K-ACh} was found to accelerate AF in humans.¹⁷ Gain of function in I_{Ks} due to

mutations in *KCNQ1*¹⁸ and *KCNE2*^{19,20} genes were found to be associated with familial AF. The common pathway in these studies as well as in SQT patients is the increase in K^+ conductance in the atrium abbreviating APD and ERP. Our study provides evidence in support of the hypothesis that amplified atrial heterogeneity leading to SDR contributes to the development of AF in SQTs.

Transmural dispersion of repolarization (TDR) has been shown to exist in the canine right atrium and to play a role in arrhythmogenesis.^{21, 22} It is possible that accentuation of atrial TDR could have contributed to the development of atrial arrhythmias in our investigation. We did not record transmural dispersion of repolarization in the current study because of technical difficulties (one has to flip the preparations over several times during the course of an experiment in order to accomplish this).

It is not clear why PD-118057 caused a greater abbreviation of repolarization in PM compared to CT. The densities of I_{Kr} and I_{Ks} are similar in canine PM and CT²³ and the extent of APD prolongation induced by I_{Kr} inhibition in both PM and CT is similar as well.²²

It is noteworthy that whereas AF is often initiated by rapid firing foci arising from the pulmonary veins taking advantage of a substrate in the left atrium, in other cases it has been shown that the trigger and substrate for AF may arise from different regions of either atria.^{24–26} The present study provides evidence in support of the thesis that AF can arise from a substrate and trigger located exclusively in the right atrium.

Isoproterenol alone did not induce sustained AF in any of our preparations. However, when added in the presence of the I_{Kr} agonist PD-118057, we were able to induce sustained AF. Our results are in accord with previous studies demonstrating that sympathetic stimulation by itself is not very effective in inducing AF,^{27,28} but that it plays a role in the maintenance of AF²⁸. Our study suggests that adrenergic interventions or hyper-adrenergic states may place SQTs patients at higher risk for life-threatening arrhythmic events.

Our experimental model also provides insight into the optimal approach to therapy in the management of AF in SQTs and the mechanistic foundation for this approach. Quinidine was found to be most effective in prolonging ERP and in preventing the induction of AF, due to its combined actions to inhibit I_{Kr} and I_{Na} . This combination of ion channel effects has recently been highlighted as an effective atrial-selective approach in the management of AF.^{29–31} Gaita et al.¹³ demonstrated the efficacy of quinidine in patients with SQTs. Hydroquinidine caused QT prolongation, increased ventricular ERP and rendered VF non inducible, whereas the Class IC and III antiarrhythmic drugs failed to do so. Moreover, quinidine also restored QT-RR relationship towards the normal range.³² In a one year follow up, patients treated with hydroquinidine remained asymptomatic and no further episodes of ventricular arrhythmia were detected. Although the effectiveness quinidine has only been demonstrated in SQT1, there is reason to believe that it may be effective in the other forms of SQTs. Prolongation of QT interval by quinidine has been reported in one patient with SQT4.³³ In contrast, I_{Kr} blockers such as sotalol were not effective.¹³ I_{Kr} antagonists were also not effective in preventing and terminating vagally-mediated AF in dog³⁴ or in a goat AF model produced by prolonged atrial tachypacing.^{35, 36} During a short action potential, fewer channels are activated and therefore I_{Kr} blockers may be less effective.³⁵ This may in part explain why E-4031 in our model as well as sotalol in SQT patients does not prevent induction of arrhythmias. Lidocaine, an I_{Na} blocker was also not effective in preventing induction of atrial arrhythmias in our model as in clinical AF.³⁷ Despite restoring ERP to normal, atrial arrhythmias were still inducible after lidocaine (although not as easily as before), suggesting that dispersion of repolarization may be more important than APD in the generation of AF. Only blockade of both potassium and sodium currents prevented the induction of AF. Thus, the wedge model not only mimics the clinical

syndrome in its ability to produce atrial arrhythmias, but is also consistent with the efficacy of anti arrhythmic therapy in the clinic.

It is noteworthy that the lack of an effect of sotalol in SQT1 cases associated with an N588K mutation in *KCNH2* is due to the fact that the gain of function in I_{Kr} current caused by the missense mutation also desensitizes the channel to traditional I_{Kr} blockers such as sotalol. Heterologous expression studies have shown that the N588K mutation in *KCNH2* dramatically increases I_{Kr} density and reduces the affinity of the channel to d-sotalol by 20-fold.⁷ Similarly, the affinity of E-4031, was shown to be reduced.³⁸ The reduced sensitivity to the I_{Kr} blockers is attributed to the +90 mV shift in the voltage-dependence of inactivation of human ether-a-go-go-related gene (HERG) channels.³⁹ The inactivated state of the channel normally stabilizes the interaction of the channel with most I_{Kr} blockers. Thus, failure of rectification of current due to loss of inactivation of the channel renders the I_{Kr} blockers in N588K *KCNH2* channels largely ineffective.^{7, 38, 39} Consistent with this observation, heterologous expression showed that affinity of mutant channel for an open-state channel blocker like quinidine is relatively high.^{32,38} Indeed, the N588K mutation reduces the affinity of quinidine for the I_{Kr} channel by only 5.8-fold.

Limitations of the Study

Drug-mediated accentuation of I_{Kr} , although capable of recapitulating the electrophysiologic and arrhythmic manifestations of the SQT1, may not produce a change in I_{Kr} qualitatively equivalent to that encountered in all forms of congenital SQT1. Of note, a specific genetic model of the disease would have the same limitation in that it would not be representative of other genetic forms of the syndrome.

Extrapolation of our results obtained from *in vitro* animal models to the clinic or to the understanding of *in vivo* phenomenon must be done with great caution. The absence of exogenous autonomic modulators and other hormones absent in isolated Tyrode's perfused preparations may influence the results.

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Abbreviations

AF	atrial fibrillation
APG	Atrial Appendage
APD	action potential duration
APD ₇₀	Action potential duration measured at 70% repolarization
APD ₉₀	Action potential duration measured at 90% repolarization
AT	atrial tachycardia
AVR	atrio-ventricular ring
CT	crista terminalis
DF	dominant frequency

DMSO	Dimethyl Sulfoxide
ECG	electrocardiogram
ERP	effective refractory period
FFT	Fast Fourier transform
HERG	human ether-a-go-go-related gene
$I_{Ca,L}$	L- type calcium current
I_{K-Ach}	acetylcholine-activated potassium current
I_{K1}	inward rectifier potassium channel current
I_{Na}	sodium channel current
Ito	transient outward current
IVC	Inferior Vena Cava
PM	pectinate muscle
RA	right atrium
SCD	sudden cardiac death
SD	standard deviation
SDR	spatial dispersion of repolarization
SQTS	short QT syndrome
SVC	Superior Vena Cave
TDR	Transmural dispersion of repolarization
VF	ventricular fibrillation

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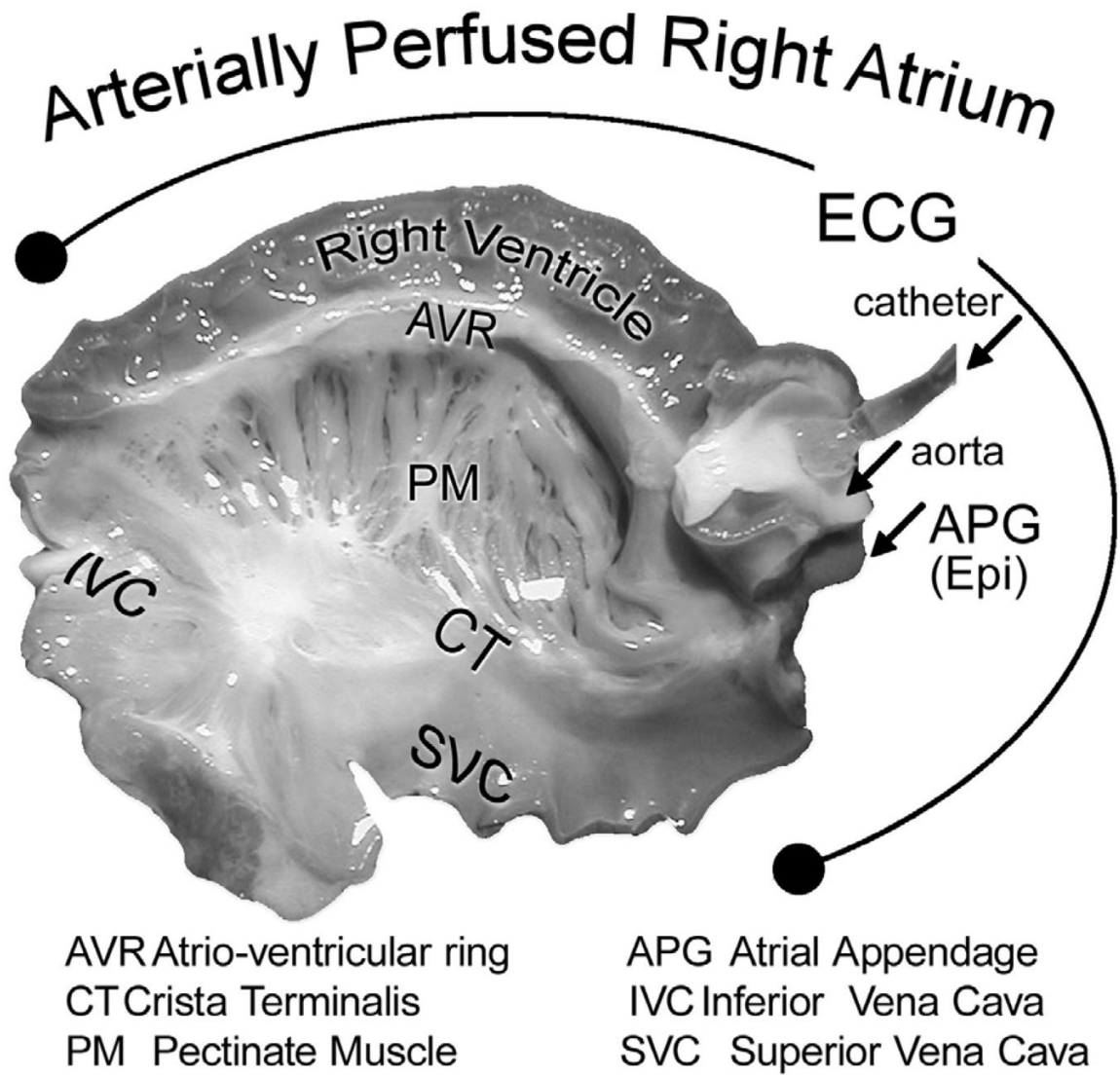


Figure 1.
 Unfolded isolated canine coronary-perfused right atrial preparation.

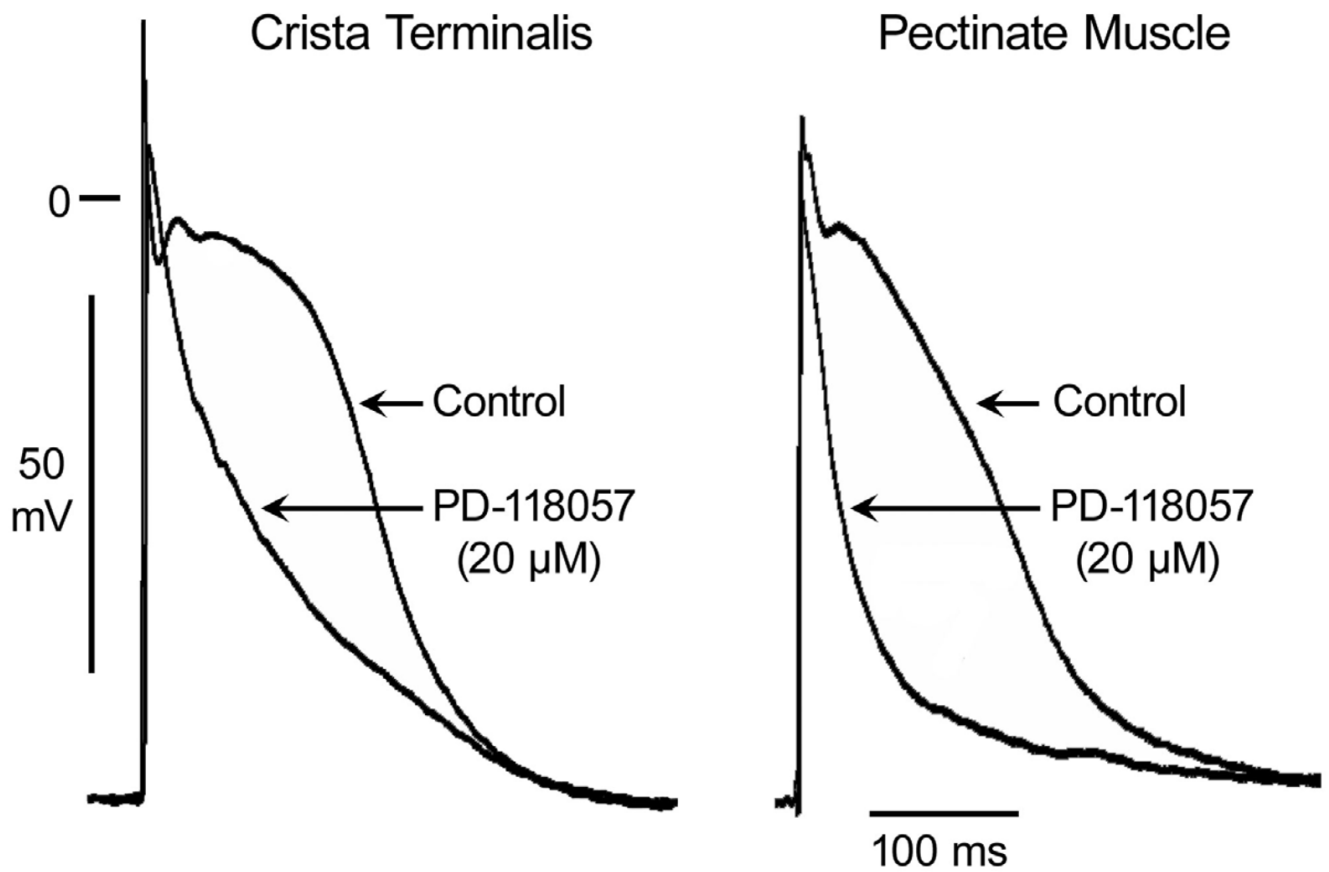


Figure 2. Heterogeneous abbreviation of action potential duration (APD) by I_{Kr} agonist PD-118057 (20 μ M) in coronary-perfused canine right atrium. PD-118057 produced a greater abbreviation of APD in pectinate muscle than in crista terminalis.

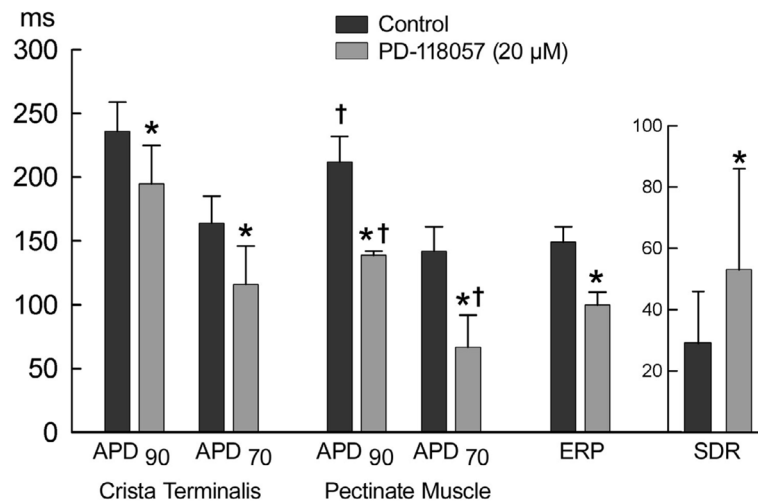


Figure 3.

Composite data of the effect of PD-118057 (20 μM) to abbreviate effective refractory period (ERP) and differentially abbreviate action potential duration (APD) measured at 70 and 90% repolarization (APD₇₀ and APD₉₀) of pectinate muscle (PM) and crista terminalis (CT), thus increasing spatial dispersion of repolarization (SDR) in all 28 preparations. Reported ERP was recorded from PM region. * PD-118057 vs. respective control, $p < 0.05$

† vs. respective CT APD value, $p < 0.05$

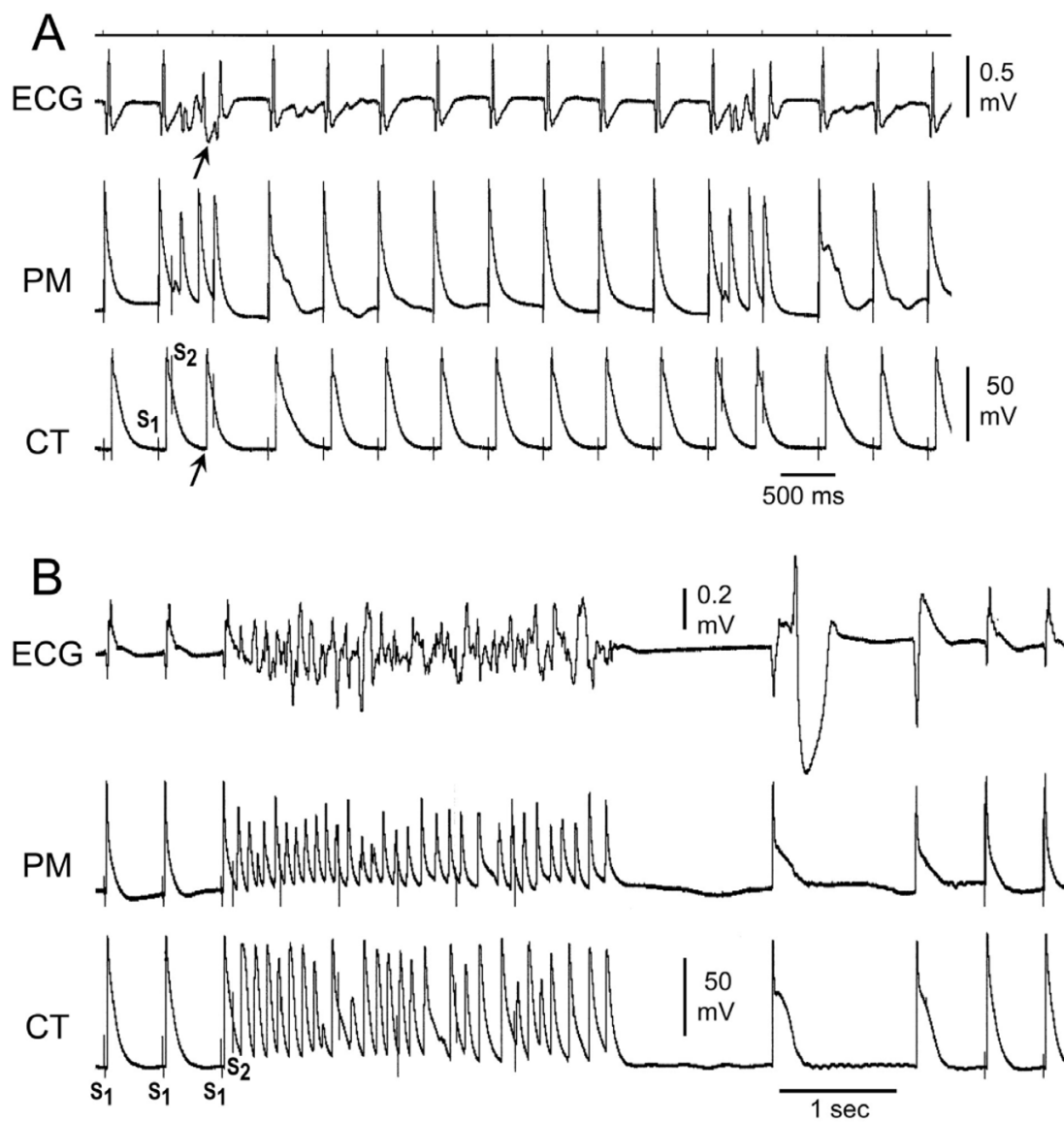


Figure 4.

A: PD-118057 induces a greater abbreviation of action potential duration (APD) in pectinate muscle (PM) than in crista terminalis (CT), facilitating the induction of atrial fibrillation (AF). A single extrastimulus applied to the pectinate muscle region gives rise to a response in PM but not CT, resulting in the generation of a single reentrant extrasystole (arrow). **B:** With further exposure to PD-118057, a single premature stimulus to the pectinate muscle region of same arterially-perfused canine atrial preparation leads to induction of atrial fibrillation (AF). Once again, the I_{K_r} agonist is observed to abbreviate APD of PM more than that of CT.

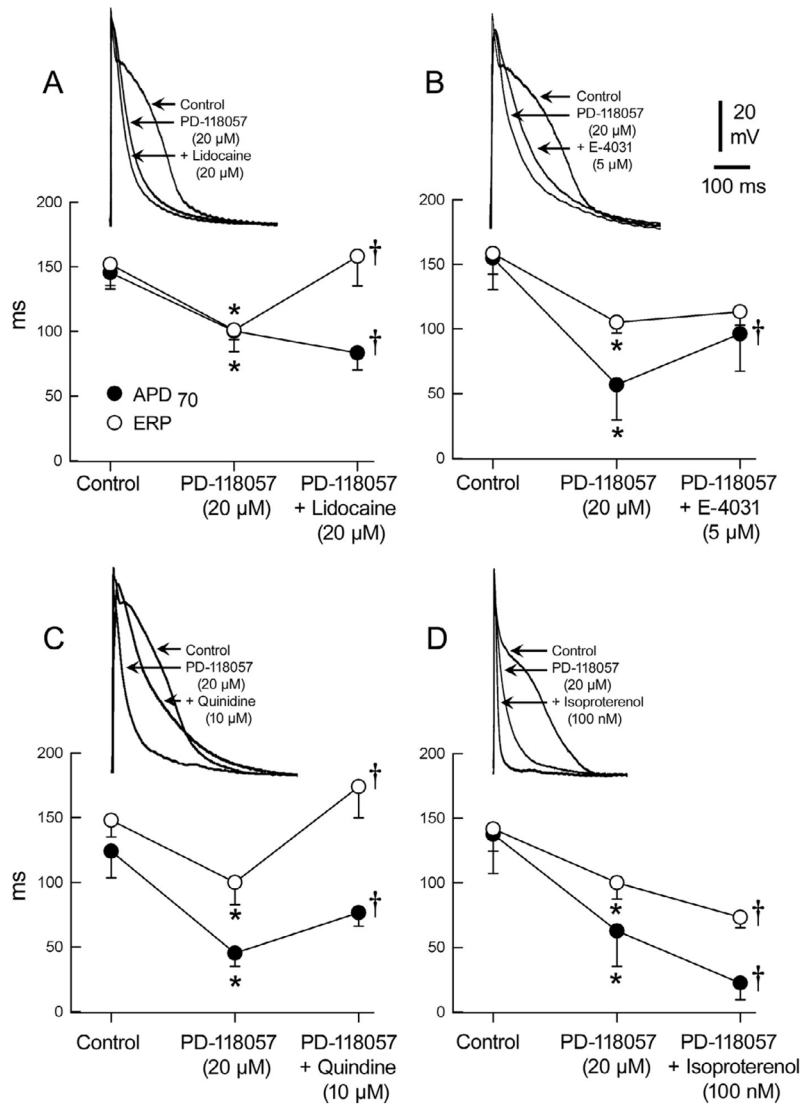


Figure 5. Effects of PD-118057 on action potential morphology and effective refractory period (ERP) recorded from the pectinate muscle region of the arterially-perfused canine atrial preparation and the effect of various antiarrhythmic drugs. Upper traces show representative traces and graphs below present the composite data with each drug. **A:** Lidocaine (20 μM) did not prolong APD but significantly prolonged ERP. **B:** Although E-4031 (5 μM) prolonged APD and ERP, it lacked the ability to reverse APD and ERP back to control conditions. **C:** Quinidine (10 μM) prolonged both APD and ERP, but only ERP reversed back to control conditions. **D:** Isoproterenol (100 nM) accentuated the effect of PD-118057 (20 μM) further abbreviating APD₇₀ and reducing the effective refractory period (ERP). * PD-118057 vs. control; $p < 0.05$ †Lidocaine/E-4031/Quinidine/Isoproterenol vs. PD-118057; $p < 0.05$

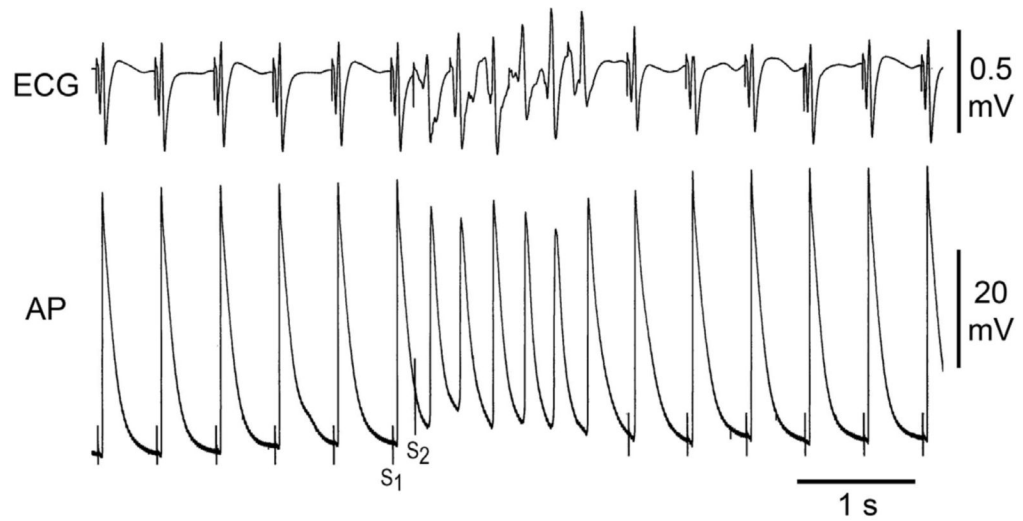


Figure 6. Lidocaine (20 μ M) added to PD-118057 containing coronary perfusate prevented AF, but permitted induction of short episodes of atrial tachycardia with a single premature stimulus to the pectinate muscle region.

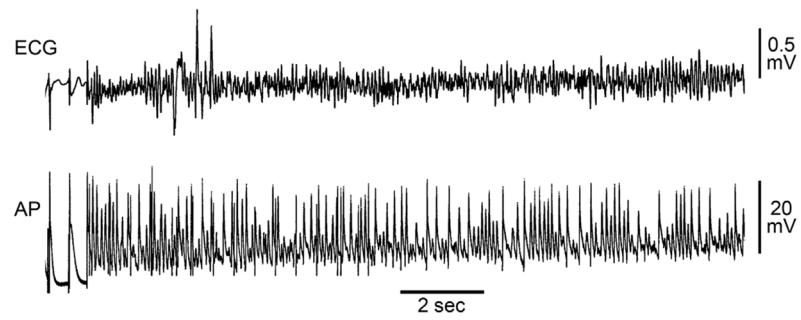


Figure 7. Isoproterenol (100 nM) in the presence of PD-118057 (20 μ M) permits induction of very rapid sustained (>30sec) atrial fibrillation (AF).

Table 1

Effect of lidocaine, E-4031, quinidine and isoproterenol on atrial electrophysiological parameters and atrial fibrillation (AF) inducibility in an SQT1 model of AF.

	Lidocaine (20 μM) (n=5)		E-4031 (5 μM) (n=5)		Quinidine (10 μM) (n=5)		Isoproterenol (100 nM) (n=6)	
	Control	PD-118057 (20 μM) +Lidocaine	Control	PD-118057 (20 μM) +E-4031	Control	PD-118057 (20 μM) +Quinidine	Control	PD-118057 (20 μM) +Isoproterenol
APD ₇₀ CT (ms)	187±13	126±17*	170±16	146±26 [†]	171±24	96±42*	160±36	110±51*
APD ₇₀ PM (ms)	145±12	100±15*	154±24	96±28 [†]	124±20	45±10*	137±30	62±27*
ERP PM (ms)	152±16	101±7*	158±16	113±10 [†]	148±13	100±17*	141±17	100±12*
AF/AT	0/5	5/5 (AF)	0/5	4/5 (AF)	0/5	5/5 (AF)	0/6	6/6 (AF)

APD70: Action potential duration measured at 70% repolarization, AF: atrial fibrillation, AT: atrial tachycardia, CT: crista terminalis, ERP: effective refractory period, PM: pectinate muscle

* PD-118057 vs. control; p< 0.05

[†] Lidocaine/E-4031/Quinidine/Isoproterenol vs. PD-118057; p< 0.05