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Arrhythmogenic substrate in deep intra-trabecular structures of RVOT endocardium in canine model of Brugada syndrome

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Abstract

Introduction: A prominent action potential (AP) notch in the epicardium (Epi) of the RVOT is known to predispose to the development of closely-coupled phase 2 reentrant extrasystoles, capable of precipitating ventricular tachycardia and fibrillation (VT/VF) in the setting of BrS. Ablation of this Epi substrate exerts an ameliorative effect. In some BrS patients, Endo ablation of the RVOT is effective as well. The prime objective of this study was to examine the electrophysiological basis for premature beats originating from the endocardium (Endo) of the right ventricular outflow tract (RVOT) in experimental models of Brugada syndrome (BrS). *Methods:* Canine coronary-perfused cardiac preparations incorporating the RVOT (n=15) were studied using standard microelectrode techniques. Terfenadine, a sodium and calcium channel blocker, was used to pharmacologically mimic the effects of the genetic defects associated with BrS. *Results:* Under baseline conditions, a prominent AP notch was recorded in Epi and in the deep intra-trabecular structures of RVOT Endo, but not in the smooth Endo surface of the RVOT. Terfenadine markedly accentuated the AP notch in the deep intra-trabecular structures of RVOT Endo leading to the development of closely-coupled phase 2 reentrant extrasystoles capable of triggering polymorphic VT/V. Still, Epi RVOT region was more likely to develop extrasystoles than Endo RVOT. VT/VF was recorded in 12/15 preparations. *Conclusions:* Our findings suggest that the deep intra-trabecular structures of RVOT Endo harbor the substrate for the development of phase 2 reentrant extrasystoles capable of triggering VT/VF. Our data may help to explain the effectiveness of Endo RVOT ablation in some BrS patients.

Arrhythmogenic substrate in deep intra-trabecular structures of RVOT endocardium in canine model of Brugada syndrome

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Short running title : Endo RVOT triggers VF in BrS

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Conclusions: Our findings suggest that the deep intra-trabecular structures of RVOT Endo harbor the substrate for the development of phase 2 reentrant extrasystoles capable of triggering VT/VF. Our data may help to explain the effectiveness of Endo RVOT ablation in some BrS patients.

Key words: Cardiac arrhythmias, Ventricular fibrillation, Extrasystole, Electrophysiology, J wave syndrome.

CONDENSED ABSTRACT

The epicardium of the right ventricular outflow tract (RVOT) has long been considered to be the source of the arrhythmogenic substrate in Brugada syndrome (BrS), accounting for the ameliorative effect of epicardial ablation. However, endocardial ablation has also been reported to be effective in some patients. Using canine RVOT preparations, we demonstrate for the first time an arrhythmogenic substrate (prominent action potential notches) in the deep intra-trabecular structures of RVOT endocardium, capable of generating closely-coupled phase -2-reentrant extrasystoles capable of triggering polymorphic VT/VF in the setting of BrS. Our findings may explain the effectiveness of Endo RVOT ablation in some BrS patients.

INTRODUCTION

A prominent action potential (AP) notch in the epicardium (Epi) of the right ventricular outflow tract (RVOT) predisposes to the development of closely-coupled phase 2 reentrant extrasystoles, capable of precipitating ventricular tachycardia and fibrillation (VT/VF) in the setting of Brugada syndrome (BrS).[1,2] These observations have provided an understanding of why ablation of the RVOT Epi exerts an ameliorative effect in patients with BrS.[3-8] However, endocardial (Endo) ablation of the RVOT has also been reported to be effective in preventing BrS-related VF in some patients.[9-12] These observations suggest that parts of Endo may also harbor a substrate contributing to arrhythmogenesis in the setting of BrS. Transmembrane action potential (AP) characteristics of the complex Endo structures of the RVOT have not been fully explored. The present investigation was designed to examine the AP characteristics of RVOT Endo in canine coronary perfused preparations under baseline conditions and in the setting of BrS.

METHODS

The investigation was performed according to the Guide for Care and Use of Laboratory Animals published by the US National Institute of Health. The study was approved by the Institutional Animal Use and Care Committee of the Lankenau Institute for Medical Research. Dogs weighing 12-20 kg were anticoagulated with heparin (200 IU/kg) and anesthetized with pentobarbital sodium (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)

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Tyrode's solution containing 8.5 mM $[K^+]_o$ and transported to a dissection tray. Preparations containing the RVOT were excised, cannulated and perfused from either right or left coronary arteries using polyethylene tubing. All severed coronary branches were ligated using silk thread. Non-perfused areas of the RVOT were dissected using a razor blade. Preparations perfused from the right coronary artery contained the entire right atrium and the rim of the right ventricle including the RVOT. Preparations perfused from the left coronary artery contained the RVOT and part of the septum.

The RVOT in the canine heart is supplied with blood from both the left and right coronary arteries. Out of 22 preparations perfused from the right coronary artery, the RVOT was relatively well-perfused (approximately 40-60% of RVOT) in 8 preparations and these were selected for use in the study. All seven preparations perfused via the left coronary artery had a relatively large proportion of the RVOT perfused (about 50-75%). Thus, 15 preparations perfused from right and left coronary arteries were used and data from these preparations were combined.

The coronary-perfused preparations were placed in a temperature-controlled bath with the endocardial surface up (8 x 6 x 4 cm) and perfused with Tyrode's solution at a rate of 8-10 mL/min. The Tyrode's solution contained (in mM): NaCl 129, KCl 4, NaH_2PO_4 0.9, $NaHCO_3$ 20, $CaCl_2$ 1.8, $MgSO_4$ 0.5, and D-glucose 5.5, bubbled with 95% O_2 and 5% CO_2. (37.0 ± 0.5^0C). The initial temperature of the coronary perfusate was 30^0C and gradually warmed to 37^0C over a period of 5-6 min. The temperature was maintained at 37^0C (Cole Parmer Instrument. Co., IL). The perfusate was warmed by circulation through a metal tube (Small Parts Inc. Miami, Fl) immersed in a bath chamber before flowing to the preparation so that the temperature of the perfusate matched that of the bath. The perfusate was delivered to the artery by a roller pump, with perfusion pressure of 40-50 mmHg throughout the experiment. An air trap was used to avoid bubbles in the perfusion line. The preparations were equilibrated in the tissue bath until electrically stable, usually 30 min, while pacing at cycle lengths (CL) of 500 to 800 ms. Basic stimulation was applied using a pair of thin silver electrodes insulated except at their tips.

Transmembrane APs (sampling rate 41 kHz) were recorded using floating glass microelectrodes (filled with 2.7 M KCl, 10-25 MΩ DC resistance) connected to a high input impedance amplification system (World Precision Instruments). The signals were displayed on oscilloscopes, amplified, digitized and analyzed (Cambridge Electronic Design, Cambridge, England) and stored on computer hard drive or CD. A pseudo-electrocardiogram (ECG) was recorded using two electrodes consisting of Ag/AgCl half cells placed in the Tyrode's solution 1.0 to 1.2 cm from the opposite ends of the preparation, thus measuring the electrical field of the preparation as a whole. AP parameters were measured from signals with the largest phase 0 amplitude in any given set of conditions.

Study Protocols: The preparations were paced at a CL of 1000 ms, using a pair of thin silver electrodes insulated except at their tips (bipolar rectangular pulses of 2 ms duration and twice diastolic threshold (DTE) intensity). Terfenadine, a sodium and calcium channel blocker (5-8 μM; [?] 1.5 hour), was used to pharmacologically mimic the genetic defects associated with BrS.[13] APs were recorded under baseline conditions and following 5-8 μM terfenadine. In order to record Epi, the preparations were flipped over. Spontaneous arrhythmias were continuously monitored using ECG recordings.

Drug: Terfenadine (Sigma-Aldrich, St Louis MO) was dissolved in ethanol as a 5 mM stock solution.

Statistics: Statistical analysis was performed using unpaired t-test. All tissue data are expressed as mean ± SD.

RESULTS

Under baseline conditions, APs from Endo were recorded from all preparations (n=15) and APs from Epi from 8 preparations. A significant spike and dome morphology of the AP was consistently recorded on the Epi surface of the RVOT in all 8 preparations in which Epi was measured (**Fig. 1** and **Table 1**). In contrast to the relatively homogeneous distribution of an AP notch (spike and dome morphology) on Epi, a marked spatial heterogeneity of spike and dome morphology was observed in the Endo of the RVOT, ranging from

none to very prominent (**Fig. 1**). An AP morphology lacking a notch was commonly recorded on the smooth surface of Endo, whereas a prominent notch was recorded deep within the inter-trabecular structures (**Fig. 1**). The RVOT Epi displayed a prominent AP notch. However, phase 0 amplitude, phase 1 magnitude, and phase 1 amplitude were larger in deep trabecular Endo vs. Epi RVOT (**Fig. 2** , Table 1). No arrhythmias were recorded under baseline conditions.

Under experimental conditions pharmacologically mimicking BrS (i.e., terfenadine-mediated block of I_{Na} and I_{Ca}; 5-8 μM; [?] 1.5 hour), our principal focus in these studies was to determine spatial differences in the AP characteristics of Endo given the fact that we had a limited period of time to collect these data prior to development of VT/VF. It is noteworthy that the characteristics of right ventricular Epi AP and phase 2-reentry in the setting of BrS are well documented.[13-15] Out of the 15 preparations studied, high quality APs were recorded from the Endo regions in 12 (due to an "early" onset of persistent VF in 3 of the preparations). Terfenadine markedly accentuated the spike and dome morphology of APs recorded from the intra-trabecular region of RVOT Endo, but caused little change in the early phases of the AP recorded from the smooth Endo surface (**Fig. 3; Table 2**). Marked heterogeneities were commonly observed in the AP notch recorded from the intra-trabecular structures of the RVOT Endo, ranging from a significant augmentation of the AP dome and delay in the onset of phase 2 to loss of the AP dome (**Fig. 4**). Under similar conditions, little heterogeneity was observed in the characteristics of APs recorded from the smooth surface of the RVOT Endo (**Fig. 4**). Accentuation of the AP notch leading to delay in the onset of phase 2 and in some cases to loss of AP dome in the intra-trabecular Endo was associated with the development of phase 2 reentrant extrasystoles (**Fig. 4**). When considering preparations in which the origin of the premature beats could be reasonably determined (based on the polarity of the QRS in the ECG traces; n=9 preparations; **Fig. 5**), an Epi and Endo origin of the extrasystole was observed in 8 and 5 preparations, respectively, with 5 of these preparations displaying both Epi and Endo origin, and 4 and 1 preparations displaying only Epi or only Endo origin, respectively.

In the setting of experimental BrS, closely-coupled extrasystoles were recorded in 14/15 preparations, giving rise to polymorphic VT/VF in 12/15 preparations. The first episode of VT/VF appeared after 93-120 minutes of perfusion with terfenadine. We observed [?] 3 self-terminating episodes of VT/VF in each of the 12 preparations and in 7 of them VT/VF was persistent ($>$ 1 hour). **Figure 6** depicts examples in which VF was initiated by extrasystoles originating from either Epi or Endo of the RVOT.

DISCUSSION

To the best of our knowledge, the present study is the first to demonstrate that the deep intra-trabecular structures of the canine Endo RVOT display APs with a prominent spike and dome morphology, that when accentuated in the setting of BrS, could lead to the development of phase-2-reentry and VT/VF.

Action potential spike and dome and phase-2 reentry

It is well established that in large mammals, including humans and dogs, transient outward current (I_{to}) density is much greater in ventricular Epi vs. Endo cells and that this is responsible for a more pronounced spike and dome morphology of the AP in ventricular Epi than in Endo.[16] Moreover, Epi of the RVOT is known to display a more pronounced spike and dome AP morphology than Epi of the rest of RV or LV.[14,17]The prominent AP notch in the RVOT Endo intra-trabecular structures is most likely due to the presence of a prominent I_{to}.

In previous studies, we and others have shown that a net outward shift in the balance of current during the early phases of AP can lead to accentuation of the AP notch, can result in loss of AP dome at some sites but not others in RV and LV Epi.[13-15] This is observed following augmentation of I_{to} or $I_{K\text{-}ATP}$ (using NS5806 or pinacidil) or following inhibition of I_{Na} and/or I_{Ca} (using terfenadine, ajmaline or verapamil).[13-15] When loss of the dome is spatially heterogeneous, it can lead to propagation of AP dome from regions in which it is maintained to regions where it is lost, resulting in a closely-coupled extrasystole due to phase 2 reentry.[13,15]

Endocardium of RVOT as a source of triggers in BrS

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The epicardial surface of the RV, particularly the RVOT, has been shown to be the site of origin of phase-2-reentry-induced extrasystoles capable of trigging VT/VF in the in ventricular wedge and whole heart models of BrS.[13-15,17-19] Consistent with these experimental observations, numerous clinical studies[3-8,12,20] as well as experimental studies[21] have demonstrated that ablation of the RVOT Epi substrate can significantly reduce the occurrence of VT/VF in the setting of BrS. Ablation of the RVOT Endo has also been shown to reduce BrS-associated VT/VF in some clinical cases.[9-12] These data suggest that RVOT Endo can be involved in the generation of VT/VF in patients with BrS.

The present study points to the presence of an arrhythmogenic substrate in the deep intra-trabecular structures of the Endo of the RVOT in the setting of BrS, similar to that previously described in the Epi of the RVOT.[22] Consequently, ablation of either surface of the RVOT has the potential to reduce BrS-related arrhythmogenicity. When applied to the ventricular endocardium, an ablation catheter is likely to ablate both the "smooth" muscle and deep intra-trabecular structures, thus exerting an ameliorative effect. An alternative explanation for the effectiveness of Endo ablation in some patients is that ablation of RVOT Endo may extend to the RVOT Epi, thus affecting Epi BrS substrate (considering that the RVOT is relatively thin).[12,23]

To the best of our knowledge, this is the first study to systematically examine the AP characteristics of RVOT Endo in the setting of BrS using intracellular microelectrode techniques. Our data suggest that the deep structures of RVOT Endo, in addition to the RVOT Epi,[14,17] can be the source of the substrate for development of phase 2 reentry, capable of triggering VT/VF in BrS. Interestingly, there are substantial electroanatomical abnormalities on the Endo RVOT surface in patients with Brugada syndrome detected with unipolar mapping, and these abnormalities correlate with VF occurrence.[24]

Limitations

Our study was performed using isolated coronary-perfused preparations and the genetic defects responsible for BrS were pharmacologically mimicked. Despite these limitations, coronary-perfused canine ventricular models have been previously shown by us and others to be capable of recapitulating the principal electrocardiographic arrhythmic clinical manifestations of BrS, as well as the response to autonomic and pharmacologic manipulation.[2,25,26]

CONCLUSION

Our finding demonstrate for the first time that the deep intra-trabecular endocardial structures in the region of the RVOT, in addition to those in the epicardium of the RVOT, harbor the substrate for BrS in the form of prominent AP notches that can serve as the source of phase 2 reentrant extrasystoles capable of triggering VT/VF.

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Table 1. Characteristics of action potentials recorded from the intra-trabecular and smooth surface of the Endo vs. those recorded from the Epi surface of the RVOT under baseline conditions

	Endocardium (n=15)	**Endocardium** (n=15)	**Epicardium** (n=8)
	Intra-trabecular	"Smooth Surface"	
Phase 0 amplitude (mV)	98±6**	99±5#	84±6
Phase 1 amplitude (mV)	71±4*+	91±5#	64±3
Phase 2 amplitude (mV)	89±5*	92±4	95±4
Phase 1 magnitude (mV)	27±7*+	8±3#	21±4
Phase 2 magnitude (mV)	18±4**+	1±1#	31±4

The AP with the most prominent spike and dome morphology recorded in each preparation was taken for statistical analysis. CL = 1000 ms. * p<0.05 vs. Epi. ** p<0.001 vs. Epi. + p< 0.001 vs Endo "smooth". # p<0.001 vs. Epi.

Table 2. Terfenadine-induced accentuation of the AP spike and dome morphology in the Endo intra-trabecular regions of the RVOT

	Intra-trabecular	**Intra-trabecular**	**"Smooth Surface"**	**"Smooth Surface"**
	Control	Terfenadine	Control	Terfenadine
Phase 0 amplitude (mV)	99±5	92±7**	100±6	95±5*
Phase 1 amplitude (mV)	71±4	67±5*	92±3	90±4
Phase 2 amplitude (mV)	90±4	95±4*	92±4	93±4
Phase 1 magnitude (mV)	28±7	25±7	7±3	5±5
Phase 2 magnitude (mV)	19±5	27±6**	1±1	3±2

The AP with the most prominent spike and dome morphology recorded in each preparation was taken for statistical analysis. CL = 1000 ms. N= 12 for each. * p>0.05 vs. control **. p>0.01 vs. control.

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Figure 1. Typical action potential (AP) morphologies recorded from the Epi and Endo surfaces of the canine RVOT. A significant AP spike and dome morphology giving rise to a prominent notch was present

throughout the Epi surface. On the Endo surface, manifestation of an AP notch ranged from very prominent to none. A prominent AP notch was recorded from the deep intra-trabecular Endo structures.

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Figure 2. Typical action potential morphologies recorded in the intra-trabecular Endo and Epi surfaces in canine RVOT. Arrows depict the difference between the Endo and Epi.

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Figure 3. Terfenadine-induced accentuation of spike and dome morphology in the intra-trabecular region of Endo. CL= 1000 ms.

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Figure 4. Dynamic appearance and disappearance of AP dome in the intra-trabecular Endo and phase-2 reentry-induced propagated extrasystole originating from the Endo of the RVOT in the presence of terfenadine. Each panel shows simultaneously recorded ECG and action potentials (AP). All recordings were obtained from the same preparation. The stimulation electrode was located on the "smooth" Endo.

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Figure 5. Endocardial and epicardial origin of extrasystoles in the setting of experimental BrS. The Epi/Endo origin of the extrasystoles were determined based on the polarity of R wave. The tracings were recorded in the same preparation.

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Figure 6. Extrasystoles originating from either Epi or Endo of the RVOT can trigger VF in the presence of terfenadine (8 μM). Origin of the extrasystole can be judged on the basis of the polarity of the R wave recorded from the pseudo-ECG. The stimulation electrode was located on the smooth surface of the Endo RVOT. The two traces were recorded from the same preparation.

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